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INFRARED SPECTROPHOTOMETRIC STUDIES ON HEMOGLOBIN AS AFFECTED BY CYANIDE, METHYLENE BLUE AND CARBON MONOXIDE

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The purpose of this investigation was to find out by means of absorption spectra whether any change in the atomic configuration of the hemoglobin molecule in the infrared region of the spectrum takes place when various reagents are added. This information will be valuable in further corroboration of interpretations concerning the mechanism of methylene blue and cyanide when added to blood.

Considerable work has been done in the visible and ultraviolet portion of the spectrum but very little in the infrared part. The only other experiments known to the writer on near-infrared absorption bands of hemoglobin are those of Hartridge and Hill (1914), who found an absorption maximum of oxyhemoglobin at about 9000 Å, and those of Eggert (1935). In the measurements of the writer, no difference between the absorption of hemoglobin and that of water around 9000 Å was found.

METHODS. The experiments[1, 2] given in this paper were done with a Bausch and Lomb spectrophotometer with camera attachment and absorption spectra photographed on Eastman infrared photographic plates.

[1] The preliminary series of experiments made with the technical assistance of Dr. Willi Cohn consisted in placing samples of sheep blood in an absorption cell and taking photographs of the density of the solution on Eastman spectrophotographic plates. These showed only qualitative results and could not be used in further interpretations.

[2] The technical assistance of Dr. L. A. Straight, Mr. L. J. Mullins and Dr. C. A. Fowler, Jr., is herewith acknowledged. Assistance as follows is gratefully acknowledged: clerical and photographic assistance made possible by WPA Project 11608 and grants made from the Ella Sachs Plotz Fund and the Board of Research of the University of California.

Curves as shown in the figures were obtained from these spectra using a microphotometer.

In these experiments a 20 per cent solution of sheep blood dissolved in 0.4 per cent NH_4OH or H_2O was used. Eastman spectroscopic plates nos. P, Q, R and Z, hypersensitized in 20 per cent NH_4OH immediately before use, and panchromatic plates were used. Each of these plates is sensitive over a specified range of the spectrum from about 6500 Å to 12,000 Å as shown in table 1.

The various derivatives of hemoglobin solutions were produced as follows:[3] To 2 cc. of blood was added one of the following: (1) KCN; 0.2 cc. of 0.02 M. (2) $NaNO_2$; 1.00 cc. of 0.2 per cent diluted in NaCl. (3) Methylene blue; 0.1 cc. of 0.1 per cent. (4) $K_3Fe(CN)_6$; 0.5 cc. of 3.0 per cent. (5) Glucose; 4.0 cc. of 5 per cent.

The final dilution was brought up to 10 cc. by adding water or 0.4 per cent NH_4OH solution in each case.

TABLE 1

NUMBER OF PLATE	WAVE LENGTH IN Å.—MAXIMUM SENSITIVITY
	Visible spectrum and red up to:
Panchromatic	6,800 Å or 7,000 Å
R Plate	7,600 to 8,400 Å
P Plate	8,000 to 9,000 Å
Q Plate	9,400 to 10,300 Å
Z Plate	10,900 to 12,000 Å

Various combinations of these solutions, using the given proportions were also made, such as blood, KCN and methylene blue; blood, $NaNO_2$ and KCN; and so on as indicated in the graphs. The exposure of the plates varied with the kind of plate used. With the visible plates a 20 per cent solution of blood was usually used at a depth of 1 cm. in the spectrophotometer for 3 to 10 seconds. With plates of longer wave lengths, a 10 per cent solution was used and the exposure in some cases was 1 hour. The tube containing the blood was cooled with a fan. Since each series of plates varied, tests were made to give exposures which would be resolved by the microphotometer into curves with sharp maxima.

Inasmuch as the photographic plates represent degrees of blackening, or transmitted light, the results are given as percent transmission, which is the reciprocal of absorption. The height of the curves is significant in the plates which represent the infrared region (P, R, Q and Z) because com-

[3] In a previous paper equivalent concentrations of reagents were added to blood (Brooks, 1935). In this paper smaller concentrations of KCN were used to approximate more nearly the conditions found in cyanide poisoning.

parable exposures could be used. In the case of the panchromatic plates which represented the visible portion of the spectrum, the light solutions could not be given the same exposure as the dark ones without producing total blackness. In these plates, therefore, the heights of the curves of solutions containing methemoglobin are not comparable with those for hemoglobin, whereas "light source" curves were given the same exposure as "hemoglobin" curves. The chief object of this paper is to show shifts in the maxima rather than density differences, so that heights of curves are not important here.

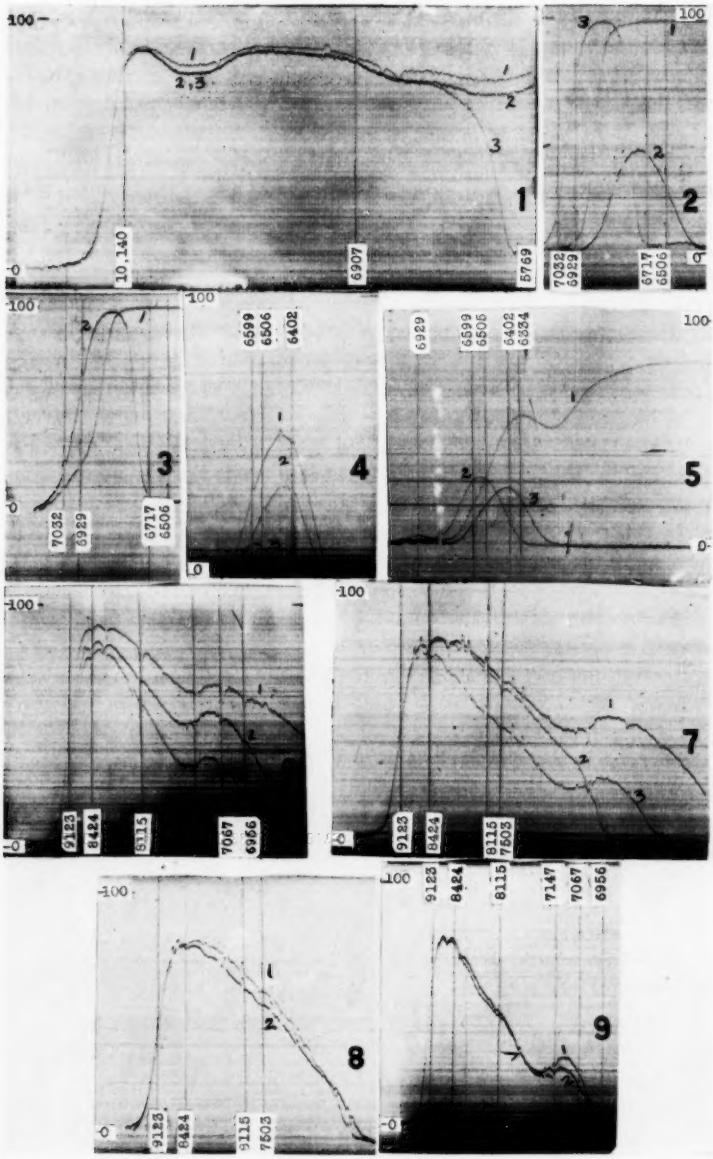
As to the dispersion comparisons, in the infrared region the spectrophotometer was adjusted to produce maximum effects. This would show differences when comparing calibration lines in different plates, but would not alter the position of absorption or transmission maxima.

Controls of the reagents alone without blood, of the light source for each plate alone, and of water, were also made. The "light source" curve represents the sensitivity of the plate itself which was determined for each plate. Calibrations of the curves with line spectra of the neon, argon or mercury arcs were also obtained according to wave length desired. Deviations in the transmission maxima from those of the appropriate controls were attributed to the reagents added to the experimental solution. When the proper density of the band spectra had been obtained, these were microphotometered and the curves so obtained are those represented in the various figures discussed below.

Abbreviations of the various solutions were as follows: CN for KCN; NO_2 for $NaNO_2$; MB for methylene blue; Hb for blood in the form of oxyhemoglobin; $FeCN_6$ for $K_3Fe(CN)_6$; "Source" for light source without any solutions.

Results. Figure 1 shows similarity of the transmission curves for source, water and blood using a Q plate which has a maximum transmission around 10,000 Å. Curves for various dilutions of blood are similar.

Panchromatic plates. These plates are sensitive from the region of visible light up to slightly over 7000 Å. Figure 2 shows the source (curve 1), that for Hb alone (curve 2), and that for Hb + NO_2 (curve 3). In the latter, the hemoglobin is converted to methemoglobin and shows the shift in the bands towards the red end. Figure 3 shows the difference between the maximum transmission for blood when CN and MB were added (curve 1) as compared with that for blood when CN and NO_2 were added (curve 2). The addition of CN in this concentration to methemoglobin (curve 2) did not produce any shift in the maximum, owing to the relatively greater concentration of $NaNO_2$ present. Nor was there any indication of an additional band such as would be expected if KCN united with methemoglobin to form a new compound. Nor was there any shift in the maximum when CN was added to blood containing MB (curve 1). Figures 2 and 3



Abscissae represent wave lengths in Å; ordinates represent per cent transmission

Figs. 1–9

were exposed on the same spectroscopic plate but on different microphotometer plates, using different ordinate scales.

Figures 4 and 5 are from the same plate, which is sensitive only to 6900 Å. Figure 4 shows that the maximum for blood and MB (curve 1) is the same as that for blood to which CN and MB had been added (curve 2), while a shift occurs in the curve for methemoglobin (curve 3) produced by adding NO_2 to blood which has stood long enough to consume the reductants. Figure 5 shows further combinations of blood, NO_2, CN and MB (curve 2) in which the maximum had again shifted to about 6590 Å from 6402 Å, as compared with curve 3 which is for blood with CN and MB added. Here again the formation of methemoglobin when NO_2 was present is to be compared with the absence of methemoglobin in spite of the combination of CN and MB.

These curves show that methemoglobin shifts the transmission maximum towards the infrared region; that blood containing methylene blue either alone or with cyanide does not cause this shift; whereas cyanide and NO_2 together in those concentrations produce no shift in the methemoglobin curve.

P-plate. This plate has a maximum sensitivity around wave length 8424 Å. Figure 6 gives the curve for source (curve 1); Hb (curve 3); and Hb and CN (curve 2). The maxima are all alike, with the primary at about 8400 Å and the secondary at about 7100 Å. The Hb curves are

Fig. 1. Showing similarity of the curves for source (curve 1), water (curve 2) and blood (curve 3). (I-Q plate #65,—1, 2, 3.)

Fig. 2. Showing difference in the curves for blood and $NaNO_2$ (curve 3) as compared with source (curve 1) and blood (curve 2). (Pan. plate #83,—1, 2, 3.)

Fig. 3. Showing difference in the curves for blood containing cyanide and methylene blue (curve 1) as compared with blood, cyanide and $NaNO_2$ (curve 2). (Pan. plate #83—4, 5.)

Fig. 4. Showing the effect of $NaNO_2$ and methylene blue on blood (which has stood) (curve 3), as compared with the lack of effect with cyanide and methylene blue (curves 1 and 2). (Pan. plate #38,—1, 3, 5).

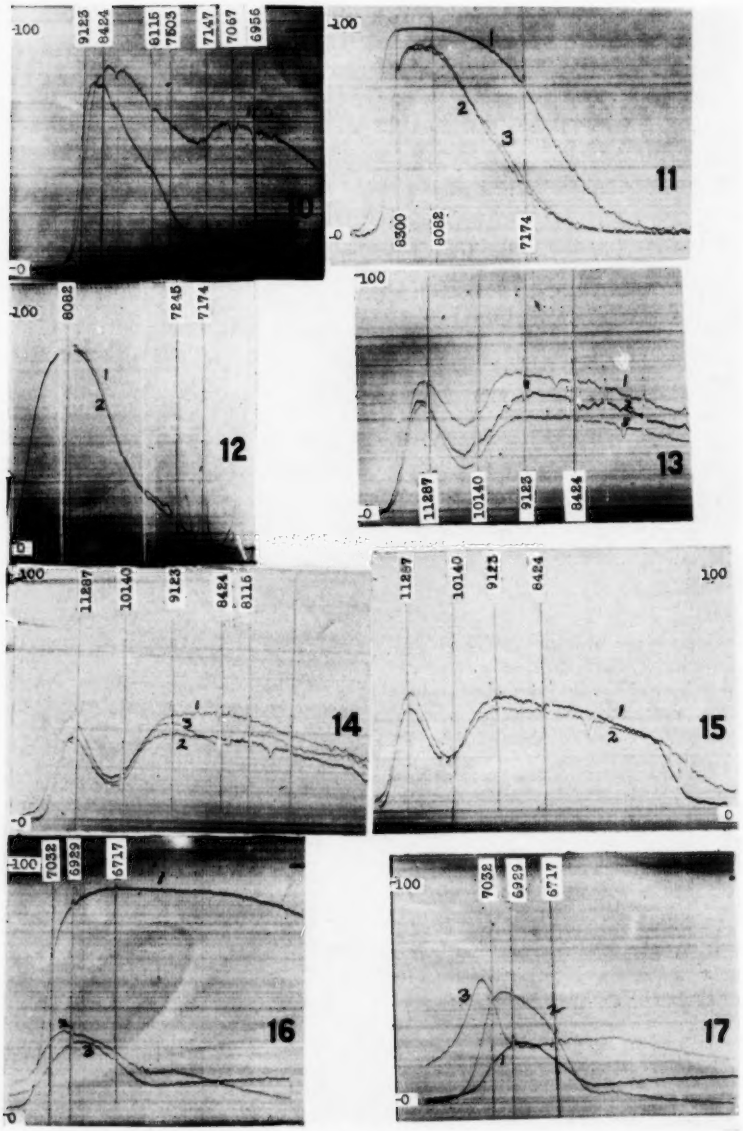
Fig. 5. Showing the absence of effect of added cyanide and methylene blue to blood (curve 3) as compared with cyanide, $NaNO_2$ and methylene blue (curve 2). Curve 3 is of methylene blue. (Pan. plate #38.)

Fig. 6. Showing similarity of the curves for source alone (curve 1), blood (curve 3) and blood containing cyanide (curve 2). (I-P plate #67,—1, 2, 3.)

Fig. 7. Showing difference in the curves for blood containing $NaNO_2$ (curve 3) as compared with blood containing cyanide and methylene blue (curve 2) and the similarity between curve 2 and curve 1, which is of blood and cyanide. (I-P plate #67,—3, 4, 5.)

Fig. 8. Showing the influence of methylene blue on fresh blood containing $NaNO_2$ (curve 2) as compared with that on blood and methylene blue (curve 1). (I-P plate #67,—7, 8.

Fig. 9. Showing similarity in blood oxidized by either $NaNO_2$ (curve 1) or potassium ferricyanide (curve 2). (I-P plate #67.)



Abscissae represent wave lengths in Å; ordinates represent per cent transmission

Figs. 10–17

slightly lower than the curve for the plate alone. Figure 7 shows the effects of NO_2 upon the primary transmission (curve 3). The maximum is shifted toward the infrared end to about 8500 Å, whereas the curve for blood containing CN and MB has the same maximum as that for blood and CN alone. The changes in the secondary maximum in the visible region due to the blue color of methylene blue are better shown elsewhere. Figure 8 shows the influence of MB on fresh blood containing NO_2 (curve 2) as compared with Hb and MB alone (curve 1). The maximum of curve 2 is only slightly shifted to the left. This can readily be seen when compared with the curve for methemoglobin of figure 7 (curve 3), all of which were photographed on the same plate. Figure 9 shows the curves for methemoglobin produced by ferricyanide (curve 1) or by NO_2 (curve 2). They are essentially identical with the primary maximum at about 8500 Å and the secondary at about 7100 Å. Figure 10 shows more plainly the shift in the maximum when pure methemoglobin produced by ferricyanide was used (curve 2) as compared with the source (curve 1).

These curves show conclusively that MB does not produce methemoglobin in the blood when CN is present.

R-plates. The sensitive region of this plate is around 8082 Å as shown by curve 1 of figure 11. There is no shift in the maximum with any of the solutions used, as shown in figure 11 and figure 12 for Hb, Hb and NO_2, and Hb and CN. Other experiments not here represented for water, NH_4OH or MB and in combinations, give similar results.

Z-plates. The region from 12,000 Å down to 8100 Å is the sensitive part of the Z-plates. There is no difference from the controls in the maximum of any of the solutions used from 11,287 Å to 9123 Å, as shown in

Fig. 10. Showing difference in the curves for blood containing K ferricyanide (curve 2) as compared with source (curve 1). (I-P plate ✱67,—1, 6.)

Fig. 11. Showing similarity in the curves for source (curve 1), blood (curve 2) and blood containing $NaNO_2$ (curve 3). (R-plate ✱81,—1, 2, 3.)

Fig. 12. Showing similarity in the curves for blood (curve 1) and blood with cyanide (curve 2). (R-plate ✱54.)

Fig. 13. Showing similarity in the curves for source (curve 1), NH_4OH (curve 2) and blood (curve 3). (I-Z plate ✱69,—1, 2, 3.)

Fig. 14. Showing difference in the curves for blood and $NaNO_2$ (curve 3) as compared with blood (curve 2) and blood containing cyanide (curve 1). (I-Z plate ✱69,—3, 4, 5.)

Fig. 15. Showing similarity in the curves for blood (curve 1) and blood containing methylene blue (curve 2). (I-Z plate ✱69,—3, 7.)

Fig. 16. Showing the sensitivity of the method in which 5 per cent methemoglobin had been added to blood (curve 2) as compared with curves for oxyhemoglobin (curve 3) and source (curve 1). (Pan. plate ✱85,—1, 3, 5.)

Fig. 17. Showing the sensitivity of the method. Curve 1, blood; curve 2, hemoglobin + 5 per cent methemoglobin; curve 3, 100 per cent methemoglobin. (Pan. plate ✱85,—3, 6, 9.)

figure 13, which includes curves for the source (curve 1), NH_4OH (curve 2) and blood (curve 3). Figure 14 shows that blood containing NO_2 (curve 3) has a maximum at 9123 Å and differs also from that of Hb alone (curve 2) around 8400 Å. This is shown better on the P-plates, and on the panchromatic plates towards the violet end. Curve 1 (Hb + CN) has the same type of curve as that for Hb alone. Figure 15 shows curves for Hb (curve 1) and for Hb and MB (curve 2). Curve 2 should be slightly shifted to the right so that the calibration line 11,287 Å coincides with the same line on curve 1. This error was made in making the microphotograph. The two curves are essentially similar in this region of the spectrum and show no such differences as are found with NO_2. This again shows that methylene blue does not produce methemoglobin in the blood under these conditions.

Figures 16 and 17 are controls with panchromatic plates to indicate the sensitivity of the method. Figure 16 shows the shift in the maximum towards the red end when 5 per cent methemoglobin was added to a solution of oxyhemoglobin (curve 2) as compared with the source (curve 1) and that for oxyhemoglobin (curve 3). Figure 17 shows the difference between a solution containing 5 per cent methemoglobin in 95 per cent oxyhemoglobin (curve 2) and oxyhemoglobin (curve 1) and methemoglobin (curve 3). Methemoglobin was prepared in the usual way ($NaNO_2$) and added to oxyhemoglobin immediately before taking the picture so that minimum reduction of the methemoglobin occurred. These curves show appreciable differences.

Discussion. These experiments show that in certain regions of the infrared spectrum there is a shift in the transmission maximum of blood towards the longer wave length when $NaNO_2$ is added alone or in combination, but that other reagents used here produce no shift. This confirms the writer's previous conclusions from studies of blood in the visible spectrum, indicating that neither cyanide nor methylene blue produces any changes in the prosthetic group of the hemoglobin molecule under the conditions used.

The spectrophotometric measurements and other experiments made by the writer (M. M. Brooks, 1932, 1933, 1934, 1935a, 1935b, 1939) have demonstrated that *methylene blue does not produce methemoglobin in the blood of living animals, but on the contrary changes methemoglobin to oxyhemoglobin* (M. M. Brooks, 1935, p. 170).[4] Since there is no evidence of the presence of methemoglobin formation by methylene blue when used in cyanide poisoning, this mechanism cannot be used in interpreting its

[4] Recently Wendel (1937) has confirmed the writer's experiments, reversing his opinion of 1933 and 1934, and Hartman, Perley and Barnett (1938) have also confirmed that methylene blue not only prevents the formation of methemoglobin but reduces it after it is formed.

action. The presence of glucose and other reductants in the blood stream of living animals is an important factor in preventing the formation of methemoglobin. This has been previously discussed by Brooks (1940) as due, in part, to the lowered redox potential produced by these substances.

Warburg (1931) states that the poisoning effect of cyanide is upon the respiratory enzyme "inactivating" it. From the experiments of the writer it is deduced that "inactivation" means a change in the electrode potential of the system involved to a negative value where Fe^{++} of the prosthetic group remains in the bivalent form and cannot shift back to the trivalent form; and furthermore, that "recovery" means a shift in the potential back to the normal range when substances are added whose systems have suitable potentials so that the system $Fe^{++} \rightarrow Fe^{+++}$ again becomes appreciably reversible (Brooks, 1940a). Such substances are methylene blue, $NaNO_2$, or any other nontoxic oxidant. The formation of methemoglobin by $NaNO_2$ is merely a by-product as in the case of sulfanilamide therapy (Brooks, 1940b). The effect of cyanide is upon the oxidation-reduction potential of the respiratory enzyme.

It is suggested from these experiments that recovery from cyanide poisoning is limited to effects upon the respiratory enzyme without invoking the methemoglobin[5] theory. Recovery from cyanide poisoning is merely a shift back to normal in the electrode potential of the respiratory enzyme system, produced by either methylene blue or $NaNO_2$ or any other similar weak, nontoxic oxidant (Brooks, 1940a).

These experiments were done to explain the mechanism of poisoning and recovery in animals by cyanide and to show that the objections to the use of methylene blue therapy[6] in carbon monoxide poisoning are not justified.

SUMMARY

Infrared spectrophotometric and microphotometric curves of blood as affected by cyanide, $NaNO_2$, or methylene blue in the presence or absence of reductants are given:

a. $NaNO_2$ produces a shift in the transmission maximum from 6402 Å to 6590 Å and from about 8400 Å to about 8500 Å.

b. Methylene blue does not produce any shift unless the reductants are used up.

c. KCN does not produce any shift either with oxyhemoglobin or with methemoglobin, in the concentrations used.

[5] Barron, 1929; Barron and Harrop, 1930; Barron and Hoffman, 1930; Anson and Mirsky, 1932; Drinker, 1938; Harrop and Barron, 1928; Henderson, 1938; Wendel, 1933; Hug, 1933.

[6] Y. Henderson, Adventures in respiration (1938); Cecil K. Drinker, Carbon monoxide asphyxia (1938).

Therefore it is again corroborated that methylene blue does not produce methemoglobin in fresh blood. In the light of the new findings and deductions it is concluded that cyanide does not attach to the prosthetic group of either ferrous or ferric hemoglobin, but acts rather by stabilizing the electrode potential of the $Fe^{++} \rightarrow Fe^{+++}$ system of the respiratory enzyme at a negative value where it must remain in the ferrous form and cannot shift back to the ferric state.

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CORONARY REFLEX DILATATIONS ACCOMPANYING CONTRACTIONS OF VOLUNTARY MUSCLES

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Physiological research literature presents an ever increasing number of observations demonstrating the close coördination of events occurring in widely separated regions of the body. We add to these lists evidence of this type of associated reactions in the skeletal neuromuscular system integrated with the autonomic nervous control regulating the flow of blood through the coronary vessels. The demonstration is the more significant in that the coördinated reactions occur in animals deprived by ether of the conscious and orderly control of the skeletal musculature.

Dogs served as experimental animals. In several hundred coronary tests of which those reported in this group are a part, the effort has been to maintain the viability of the animal in all of its complexity restricted only by the necessary use of anesthetics. By this technic one may evaluate both the passive mechanical and the active reflex factors influencing the coronary system. Ether was supplemented at times by certain hypnotics and narcotics. It is necessary to maintain a regular and strong heart beat with a vigorous blood pressure if one is to obtain orderly contractions of skeletal muscles.

If etherization is guardedly reduced to a light level it is possible, long before consciousness occurs, to secure reflex and more or less regular movements of the muscles of the legs and chest. With meticulous ether control such movements can be maintained for many seconds. During muscular activity cardiac and coronary reflexes also occur, as shown in the experimental records and data tables presented below.

Two examples are given in detail to illustrate this type of associated functions. In animal I, test 10, the first skeletal muscular contractions were moderately vigorous, somewhat irregular, and were allowed to continue for 65 seconds. The contractions were initiated without any visible external stimulus. They slowly decreased and ceased upon deeper etherization. The preliminary blood pressure was 154 mm. Hg and the heart rate 120 per minute. The new and unique feature of the test was the simultaneous development in the autonomic system of a pronounced reflex affecting the coronary system.

Protocol from dog 1, experiment 10. Weight 6.8 kilos; right vagus cut at the jugular foramen and degenerated for 12 days, all other nerves intact; chest opened and artificial respiration; intravenous heparin 30 mgm. per kilo body weight.

During controlled light etherization skeletal muscular movements of the legs and chest occurred. This reaction was associated with a pronounced coronary dilatation. There was an immediate slowing of the heart rate which was accelerated slightly after the contractions of the skeletal muscles ceased. There was some irregularity of blood pressure. The respiratory rhythm was increased and the movements augmented. The coronary dilatation began 23 seconds after the first muscular movement. The dilatation was abrupt and prolonged,—lasting 300 seconds.

It is instructive that the heart rate during the period of muscular movement was slower than the preceding normal by 15 per cent during the first 5 seconds, and still by 3 per cent at 60 seconds. Under deeper etherization the rate accelerated to 15,

TABLE 1

Data table from experiment 10, dog 1

TIME	BLOOD PRESSURE	PER CENT OF CHANGE	HEART RATE PER MINUTE	PER CENT OF CHANGE	FLOW FROM CORONARY SINUS	PER CENT OF CHANGE
	mm. Hg					
Normal	154		120		53	
15 seconds later	142	−8	102	−15	53*	Still normal
40 seconds later	173†	12	108	−10	80‡	51
60 seconds later	166	8	114	−3	91	72
90 seconds later	140	−9	138	15	69	30
140 seconds later	144	−7	144	20	64	21
175 seconds later	143	−7	156	30	62	17
240 seconds later	144	−7	144	20	56	5
300 seconds later	144	−8	144	20	50	−6
340 seconds later	144	−8	144	20	56	5

* Coronary flow still normal at 15 seconds.

† Blood pressure irregular at this time.

‡ Coronary dilatation began 23 seconds after first muscular movements.

20 and 30 per cent, measured at 90, 140 and 175 seconds. The last two minutes the rate was constant at 20 per cent over the initial heart rate.

The coronary response was a primary dilatation that began about 23 seconds after the first muscular movements. The dilatation rapidly mounted to a 72 per cent increase at 60 seconds, measured by the increase of flow of blood from the coronary sinus. The dilatation slowly disappeared until the initial normal flow was reached in 300 seconds. The form of the curve of rapid development and of slow decline of the dilatation is very typical of smooth muscle response. The rate of coronary flow was independent of, i.e., did not follow, the curve of variation of either blood pressure or heart rate. One concludes that the coronary dilatation is an independent function carried out by its own reflex nerve controls, though initiated in close association with the voluntary muscular movements. The coronary dilatation began while the heart rate was 10 per cent slower than normal. The volume of the initial coronary dilatation was rapidly increased and out of all proportion to the fluctuations of blood pressure. In fact the blood pressure is relatively constant and lower than the preactivity normal except at the 40 and 60 second periods.

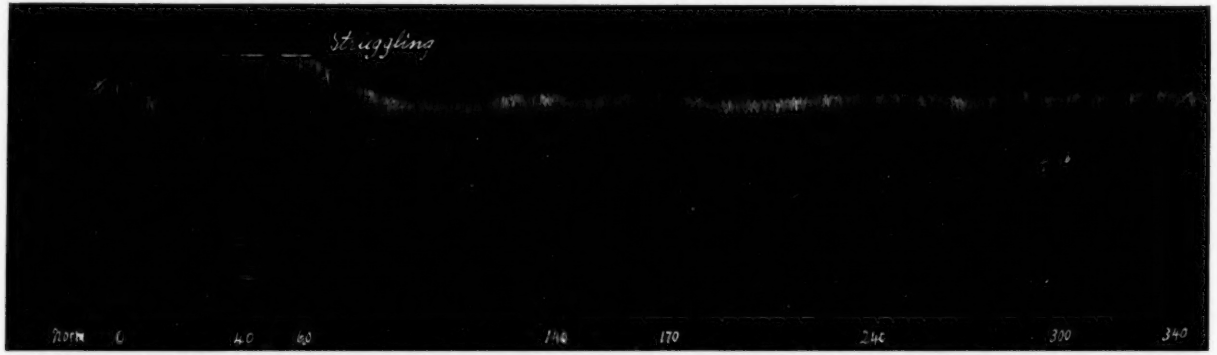

Fig. 1. Coronary dilatation associated with muscular movements under light, or sub-surgical, etherization. The top line records signals, the second line blood pressure by mercury manometer, the third measures the rate of blood flow from the coronary sinus by the author's volume manometer, time in 5 seconds and minute intervals. The numerals indicate time in seconds from the initial movement. The figure is reduced to 42 per cent of the original.

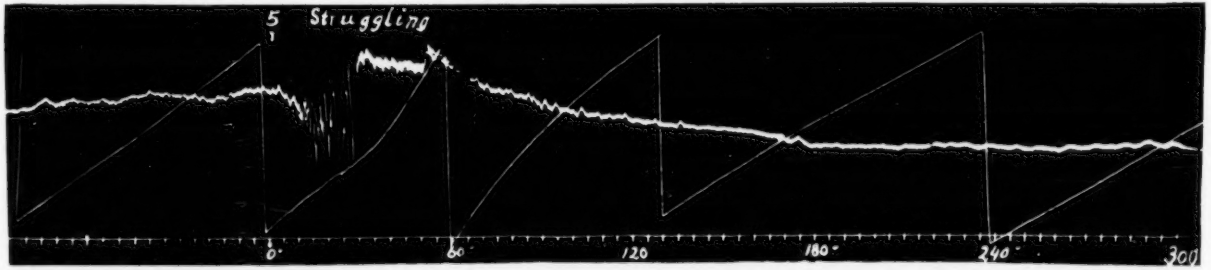

Fig. 2. A second example of response of the coronary blood vessels to reflexes associated with contractions of skeletal muscles. The legends of figure 1 apply also to this figure.

The coronary and the cardiac innervation paths of this animal are all intact except for the right vagus nerve. The cardiac slowing obviously takes place by reflexes through the left vagus. No coronary constrictions occur, hence the coronary control is not modified by the degeneration of the right vagus. All efferent dilator nerves are intact hence the dilatation is the terminial event of nerve reflexes reaching the coronary vessels by way of the thoracic and cervical pathways. This experiment demonstrates that the coronary reflexes associated with developing activity of skeletal muscles are primarily dilator and not constrictor in type. This proof is of great significance and should be emphasized.

TABLE 2

Data table experiment 5, dog 2

TIME	BLOOD PRESSURE	PER CENT OF CHANGE	HEART RATE PER MINUTE	PER CENT OF CHANGE	CORONARY FLOW; CORONARY SINUS	PER CENT OF CHANGE
	mm. Hg					
Normal	105.5		156		50	
5 seconds after	106.3	−1	144	−8	52.5	5
15 seconds after	85	−19	54	−65	49	−2
30 seconds after	91	−14	102	−35	45.5	−9
40 seconds after	124.5	18	150	−4	85	70
50 seconds after	121	15	144	−8	100	100
55 seconds after	131	24	150	−4	105	110
65 seconds after	115.5	9	156	0	100	100
75 seconds after	105	0	162	4	79.5	59
90 seconds after	97.3	−8	171	9	65	28
105 seconds after	89.5	−15	180	15	53	6
120 seconds after	84	−20	174	11	42.5	−8
135 seconds after	79.5	−25	174	11	42.5	−15
150 seconds after	77	−27	174	11	40.5	−20
165 seconds after	72	−32	174	11	37.5	−25
180 seconds after	66	−38	171	9	34	−31
195 seconds after	64	−39	168	8	33.5	−33
210 seconds after	63.2	−40	168	8	33.5	−33
240 seconds after	64	−39	168	8	36	−28
270 seconds after	63	−40	171	9	36	−28
300 seconds after	64	−39	162	4	37	−26
360 seconds after	63	−40	168	8	34.4	−31

A second experiment of like import runs more or less parallel with that given above, but a greater disturbance occurs. In this second animal the entire nervous system was intact. The observation occurred early in a long series of other tests, when the physiological mechanisms were more sensitive than later. The records and tabulated data are offered herewith.

Protocol of dog II, test 5. Weight 5.5 kilos, all nerves intact; ether; heparin; open chest, artificial respiration.

Under light etherization independent movements of skeletal muscles developed lasting 55 seconds. The data from this test are given fully in table 2 above.

Blood pressure was very irregular from the onset of the test and during muscular contractions. It decreased during the early period of 30 seconds then suddenly increased 18 per cent above the initial normal, at the 40 second period. After the contractions were controlled by ether the pressure was not sustained at a constant level, as in test 1, but continued to fall slowly for 5 minutes before reaching a constant level.

There was profound slowing and irregularity, i.e., reflex inhibitory slowing, of the heart rhythm through the first 40 seconds, then a return to the preceding normal followed by a mild acceleration as the etherization was increased.

The coronary flow was passively lowered a bit during the greatest decrease of blood pressure but at the 35 second period a sharp and active dilator reflex began. The coronary dilatation reached a maximum of 110 per cent at 55 seconds. It returned to the normal between 105 and 120 seconds. In the after period the flow gradually decreased to its lowest level at minus 31 per cent after 360 seconds, then it began again to dilate. The acute dilatation in this test was also primary, as in animal I. The coronary response bears all the characteristics of a reflex autonomic dilatation. It has the significance of a compensating reflex. The blood pressure passively influences the rate of coronary flow but the two phenomena are obviously not parallel functions in this experiment.

The coronary reflex dilatations in association with contractions of voluntary muscle are significant from a somewhat different point of view, viz., they are primary dilator reflexes. This fact supports earlier observations of the writer, that coronary reflexes in normal animals are primarily dilator in type (1, 2). They demonstrate that the acute coronary dilator reflexes are developed by the conditions that stimulate skeletal muscle movements.

The tests described in this paper furnish acute experiments which give the clue for the final interpretation of the facts developed by the ingenious method of the electric stromuhr measurements of coronary flow in the dog published by Essex, Herrick, Baldes and Mann (3). These authors say in discussing the profound coronary dilatations they obtained (p. 623), "The evidence indicates other influences as important as blood pressure in augmenting coronary blood flow when a dog is given successive increments in the rate of work".

It is well established that variations in blood pressure passively affect the rate of coronary flow. But for the experiments quoted above giving the effects of treadmill work on coronary flow may we offer the explanation that associated reflex coronary dilatations are in fact the "other influences" actively augmenting the coronary flow beyond the influence of the passive factor of variations in blood pressure.

SUMMARY

The observations presented in this paper and their physiological significance are condensed into the following:

1. Under light etherization skeletal muscular movements may be induced and controlled for brief periods.

2. During skeletal muscular activity blood pressure is more or less variable, with a tendency to rise in the later stage of the skeletal muscular activity and to fall in the after-period.

3. The heart rate shows a variable degree of reflex slowing at the very beginning of the movements, but quickly recovers and is accelerated in the after-period.

4. The coronary flow is sharply augmented in a primary reaction. The dilatation persists into the after-period. The coronary dilatation is an associated reflex of the greatest significance to cardiac nutrition during the added heart strain incident to skeletal muscular activity.

5. The coronary reactions were determined by the Morowitz technic and continuous measurement by the author's flow manometer.

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THE PRESENCE AND DISTRIBUTION OF HISTAMINE IN BLOOD[1]

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The development of new procedures of extraction has established the presence of histamine in normal blood from a number of mammalian species (1) (2). Code (3) in studying the distribution of histamine in blood concludes that the activity is associated largely (80–90 per cent) with the white cells, particularly the granulocytic elements. Although confirming Code's observations on the relatively high histamine content of the white cell layer, Anrep et al. (4) ascribe only about 50 to 60 per cent of the total histamine content of rabbit blood to this fraction, the remainder being associated with the red cells and plasma. A preliminary study (5) led us to the conclusion that, within the limits of accuracy of the methods employed, 97 to 98 per cent of the histamine content of rabbit blood is in the blood platelets. More recently an independent investigation by Zon et al. (6), utilizing the unique method of rendering rabbits platelet-free with antiplatelet serum, has led to essentially the same conclusion.

In the present communication I propose to describe a method for the extraction of blood histamine which is more rapid and less tedious than methods used at present and to elaborate on the observations previously reported.

EXPERIMENTAL PROCEDURE. Rabbits and dogs served as donors of the blood used in these experiments.

Collection of blood samples. Rabbit: Samples were obtained from the lateral vein of the ear, which had previously been shaved, made hyperemic by heating with an electric bulb, and coated with vaseline. The vein having been nicked with a razor, the blood was allowed to drop directly into the anticoagulant contained in a 15 cc. graduated centrifuge tube, extreme precautions being taken to prevent coagulation. In the majority of earlier experiments 0.07 cc. of 5 per cent sodium citrate per cubic centimeter of blood was used and made up to a volume equal to that of the blood to be obtained with calcium-free Ringer's solution. In later experi-

[1] The major portion of this paper constituted part of a dissertation for the degree of Doctor of Philosophy in Physiology at the University of Chicago.

ments, when platelet volumes were to be measured, 1.3 per cent sodium oxalate served as the anticoagulant. In a few experiments on undiluted blood heparin was employed. The tube containing the anticoagulant was chilled in an ice bath and immediately replaced after collection of the sample.

All samples of plasma used were prepared by centrifuging the blood for 20 minutes in a refrigerator room. In later experiments to avoid contamination of the plasma samples with platelets the plasma was removed from the precipitated cells and recentrifuged another 20 minutes.

Platelet-rich, cell-poor suspensions were obtained by centrifuging 10 cc. of the diluted blood (1:1) for one and one-half to two minutes, at 2,300 r.p.m. Quantitative platelet volume studies by the thrombocytocrit method of Van Allen (7) showed such suspensions to contain from 50 to 85 per cent of the platelet volume of the original diluted blood, while over 99 per cent of the red cells and 95 to 100 per cent of the white cells were absent. The platelet concentration in the centrifuged specimens was determined by diluting that quantity of suspension obtained from 1 cc. of the original blood to 5 cc. with the oxalate solution, thus making possible direct comparison of the platelet volumes in the suspension and in the original blood which is diluted 1:5 with the oxalate solution in the Van Allen procedure.

Dog: In the studies on dog blood samples were obtained by venepuncture, the syringe containing 0.5 cc. of 5 per cent sodium citrate for 10 cc. of blood. Platelet suspensions were obtained as above except that larger quantities of blood were used. Thirty cubic centimeters of blood were drawn into a syringe containing 30 cc. of 1.3 per cent sodium oxalate, mixed, and centrifuged three minutes. The pink, cloudy plasma was then removed and recentrifuged for three minutes to throw down most of the remaining corpuscular elements. After a 2 cc. sample of this suspension had been taken for a thrombocytocrit determination, 25 cc. of that remaining was centrifuged for one hour, the clear plasma decanted off, and the precipitated mass of platelets stirred up in 5 cc. of calcium-free Ringer and extracted in parallel with the whole citrate blood sample.

Hematocrit determinations were obtained on all samples used.

Methods of extraction. Electrodialysis: MacGregor and Thorpe (8) describe an electrodialytic method for the rapid extraction of histamine from tissues. Although these authors failed to detect the presence of histamine in (human) blood, the high histamine content of rabbit blood suggested the applicability of this method to blood histamine studies in this species.

Four three-chambered cells were used similar in construction to those described by MacGregor and Thorpe, except that the middle chamber was made of pure gum rubber, thus eliminating the necessity of the rubber

gaskets. Parchment paper membranes separated the middle and electrodal chambers.

Twenty-five cubic centimeters of distilled water were used in each of the electrodal chambers and 20 cc. in the middle chamber. The blood samples to be dialyzed were placed in the middle chambers of the cells, care being taken that the same equivalent quantity of electrolyte was added to each cell. The smallest samples used were 0.5 cc. blood in 2.0 cc. of isotonic electrolyte and the largest samples 5 cc. of heparinized whole blood.

The four cells were connected in parallel to a 110 v. DC source, part of the current being shunted through electric light bulbs during the initial period of dialysis. Removal of the histamine was complete within about one-half hour, although the dialysis was continued in most cases for one hour as a precautionary measure.

After completion of the dialysis the cathodal fluids were removed, chambers washed three times, the combined fluid and washings neutralized with 0.2 NHCl against phenolphthalein, and each made to 50 cc. with distilled water. The dialysates were then ready for assay or further treatment.

Trichloracetic acid extraction: As a check several experiments on rabbit's blood were conducted using the procedure described by Barsoum and Gaddum, samples being collected by allowing the blood to drop directly into weighed tubes containing the 10 per cent trichloracetic acid. Samples for dialysis were obtained at the same time.

In the dog experiments, trichloracetic acid extraction was used exclusively, since the low histamine concentration in dog blood required a quantity in excess of that which the electrodialytic cells could adequately handle.

Methods of assay. Isolated guinea-pig intestine: Routine histamine assays of the blood dialysates were made using the isolated guinea-pig intestine as the test object.

The tissue bath and heater used were essentially the same as described by Burn (9) with the exception that the Ringer-Locke solution was led through a glass coil inside the heating chamber before entering the bath, whose capacity was 25 cc. in most experiments.

It was found that the intestine obtained from a guinea pig starved 24 hours could be preserved in Ringer-Locke solution at ice box temperatures for as long as seven days with little diminution in sensitivity to histamine. After a recovery period in an aerated bath (37°C.), during which constant histamine doses produced gradually increasing contraction heights, strips refrigerated from two to seven days yielded considerably more regular and uniform responses than fresher specimens. Spontaneous activity of the isolated strip, a factor of some annoyance in this method of assay, was almost wholly absent in the preserved material. It might also be

added that the use of preserved intestinal strips renders the frequent sacrifice of costly guinea pigs unnecessary.

The standard used was a solution of histamine di-phosphate equivalent to a 0.1×10^{-6} solution of the basic amine. Only after successive checks with varying doses was the assay of any sample said to be complete. All assays have been within the limits of accuracy of ± 5 per cent, while in some experiments we feel justified in reporting an accuracy of ± 2.5 per cent.

Blood pressure of the etherized cat: In a few experiments effects of samples were compared with those of a histamine standard (1.0×10^{-6}) on the blood pressure of the etherized cat before as well as after administration of atropine.

RESULTS. *Rabbit.* Whole blood: Quantitative estimations of histamine equivalents in electrodialysates of blood from several rabbits have given a range of values varying from 1.8×10^{-6} to 5.0×10^{-6} of the basic amine for the original blood. Samples from the same rabbit taken at intervals of several days showed less variation.

1. In a series of control studies 12 samples were dialyzed in duplicate, six samples in triplicate, and four samples in quadruplicate. In all cases but one agreement was within the limits of accuracy of the assay. Redialysis of the middle chamber liquid for one hour and concentration of the resulting cathodal liquid revealed complete absence of the active substance.

2. More than 90 per cent of 5 gamma of histamine added either alone to the middle chamber or to the blood sample to be dialyzed could be recovered (six experiments).

3. The active substance was stable to prolonged boiling in NHCl.

4. Close quantitative agreement was observed between electrodialysates and trichloracetic acid extracts of duplicate samples.

5. Atropine added to the tissue bath in no way affected the final result of the assay. Hence atropine was not routinely used in these experiments.

6. Incubation of the electrodialysates with an equal volume of a 1:20 extract of dried kidney powder in phosphate buffer for 1.5 hours at 37°C. resulted in complete loss of activity. Kidney extract, as pointed out by Best and McHenry (10), is a rich source of histaminase.

7. Recently thymoxyethyl diethyl amine has been shown to abolish specifically the action of histamine on the isolated guinea-pig intestine (11). This drug was found to reduce the action of histamine and the blood dialysate on the isolated intestine to the same extent, whereas the effects of KCl and acetylcholine were altered but slightly.

8. Neither the kind of anticoagulant used (sodium citrate and sodium oxalate), nor the amount of electrolyte employed in diluting the blood seemed to have any effect on the final results. Isotonic sodium oxalate, contrary to Code's findings with solid potassium oxalate as an anticoagu-

lant (3), appeared to alter neither the total extractable histamine nor its distribution in the blood.

9. In agreement with Barsoum and Gaddum's findings (1) no significant change in the histamine content of the blood occurs on standing. In seven experiments a sample of blood dialyzed immediately was compared with a duplicate sample dialyzed after standing four hours at room temperature. In two experiments the older sample showed about 5 per cent decrease in activity. No changes occurred in samples kept for fourteen hours at ice-box temperature.

Plasma, platelets and cells: In preliminary studies, inconsistent results were obtained in the determination of the relative histamine contents of plasma and formed elements, dialysates of plasma frequently giving much higher histamine values than indicated by direct application of the plasma to the strip. Further, plasma from samples briefly centrifuged contained more histamine than plasma obtained after prolonged centrifuging. Microscopic examination of the briefly centrifuged plasma samples revealed platelets in large numbers, although the cellular elements were almost entirely absent. This fact led to the following findings:

1. In two experiments electrodialysates of a mass of platelets obtained by prolonged centrifuging of a platelet-rich, cell-free suspension possessed 65 to 75 per cent of the whole blood histamine content. The precipitated cellular elements, though washed repeatedly, showed 15 to 20 per cent of the total content with 5 per cent in the plasma. Although these experiments indicate a large portion of the histamine to be in the platelets, it was obviously impossible either to recover all the platelets by this method or to wash the cells free of platelets.

2. Using the thrombocytocrit technique of measuring platelet volumes, we could demonstrate that within narrow limits the ratio of the histamine contents of a platelet suspension and of whole blood is the same as the ratio of their platelet volumes. In five experiments the two ratios agreed to well within 10 per cent. In one experiment the difference was 18 per cent (see table 1).

3. Eight thrombocytocrit determinations in five different rabbits gave an average value of 0.85 per cent (range 0.70–1.27 per cent).

4. Application was made of the principle employed in the thrombocytocrit method of Van Allen, namely, that when blood is diluted five times with an isotonic electrolyte solution (1.3 per cent sodium oxalate) and allowed to stand, the cellular elements settle out leaving the platelets in a uniform suspension for several hours in the same concentration as existed in the blood before sedimentation. In nine experiments close quantitative agreement was found between the histamine concentrations of dialysates of unsedimented blood, the cell-free platelet suspension, and the sedimented layer of cells, in all of which the platelet concentration is

the same although the cellular contents vary widely. As stated above, dilution and standing alter neither the total extractable histamine nor the plasma histamine level (see record).

TABLE 1

Comparison between platelet volumes and histamine concentrations of whole blood and a cell-free platelet suspension

EXPERIMENT NUMBER	THROMBOCYTOCRIT (CC. PLATELETS/100 CC. WHOLE BLOOD)			HISTAMINE CONC., γ/CC.			PER CENT DIFFERENCE
	A Whole blood	B Platelet suspension	A/B	C Whole blood	D Platelet suspension	C/D	
1	0.70	0.48	1.45	5.5	3.8	1.44	1
2*	0.88	0.64	1.37	3.0	2.25	1.36	1
3	0.94	0.77	1.22	1.8	1.2	1.50	18
4	0.76	0.66	1.15	3.0	2.5	1.20	4
5	1.27	0.98	1.29	3.7	3.0	1.23	5
6	0.90	0.77	1.16	2.5	2.1	1.19	2.5

* For assay, see record.

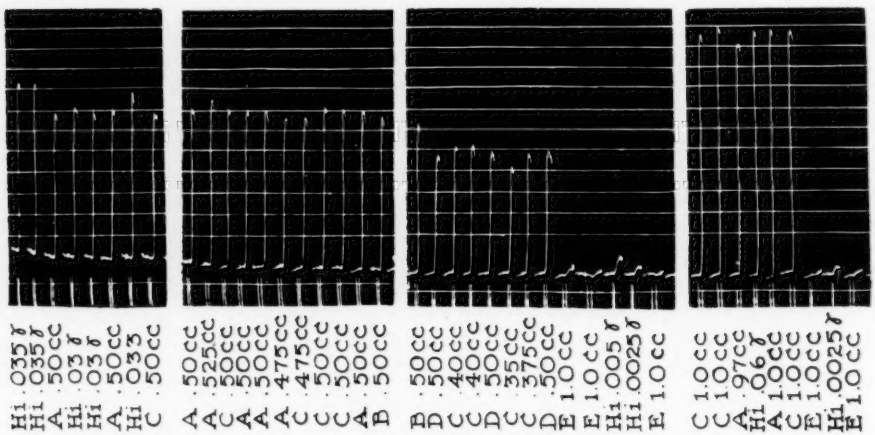

Fig. 1. Isolated guinea-pig intestinal strip, preserved four days in Ringer's solution at ice box temperature; capacity of bath 25 cc. A = whole blood, dialyzed immediately; B = whole blood, dialyzed after 3½ hours standing at room temperature; C = cell-free platelet suspension, prepared by sedimentation of cells (throm'crit = 0.88 per cent); D = cell-free platelet suspension, prepared by differential centrifugation (throm'crit = 0.64 per cent); E = recentrifuged plasma; Hi = histamine, 0.1×10^{-6} of basic amine.

Note: Each cubic centimeter of electrodialysates A, B, C and D represents 0.02 cc. of original blood or platelets therefrom; each cubic centimeter of electrodialysate E represents 0.04 cc. of plasma.

5. In fifteen experiments the average histamine concentration in plasma was 2.8 per cent of that in whole blood. In most instances the plasma dialysates were concentrated ten times before assay.

6. Electrodialysates of platelet suspensions, plasma, and whole blood, as well as trichloracetic acid extracts of whole blood, when concentrated and assayed against histamine on the blood pressure of the cat gave values which checked quantitatively to about 10 per cent of those obtained with the guinea-pig intestinal strip. The values were the same before and after administration of atropine to the cat.

Dog. Using the trichloracetic acid extraction procedure and the guinea-pig intestinal strip method for assay, we have found that in the dog, contrary to our findings in the rabbit, the platelets contain a relatively small proportion of the total blood histamine. In these experiments the values for the equivalent concentration of histamine in whole blood ranged from 0.035×10^{-6} to 0.025×10^{-6}. The corresponding histamine values for the platelets ranged from 0.7γ to 0.5γ per cc. of platelets. Since the platelet volume is about 1 per cent of the whole blood, the platelets would account only for about 15 per cent of the total histamine equivalent of the blood.

Discussion. The quantitative extraction of blood histamine in the rabbit can readily be achieved by electrodialysis in a manner more rapid and less tedious than the trichloracetic acid procedures in current use. The active substance in the blood dialysates resembles histamine in its physiochemical as well as in its pharmacological properties. Since histamine has been isolated chemically from the blood of the rabbit (12), it may be safely assumed that the active material is histamine itself.

Application of this method to studies on the distribution of histamine in rabbit blood has led to the finding that the histamine present is localized to a large extent (97–98 per cent) in the platelets. Aside from a small portion (2–3 per cent) in the plasma, which might conceivably result from unavoidable breakdown of the histamine-containing platelets, the remaining constituents of blood contribute so little to the total histamine content as to be undetectable by the methods employed.

As already indicated, these findings are at variance with those of Anrep et al. The discrepancy may possibly find explanation in the failure of these authors to prevent partial agglutination and breakdown of the platelets. Code (3) ascribes the major portion of the histamine in rabbit blood to the white cell layer of centrifuged blood (which contains most of the platelets), but suggests that the leucocytes are the histamine-containing elements.

No support has been found for the distinction raised by Tarras-Wahlberg (13) between a free-phase and a bound-phase of the histamine in rabbit plasma. From the high values he cites for the histamine content of

plasma, it seems likely that his samples were not completely free of platelets, a view which is borne out by the method he describes for obtaining plasma. Zon and his collaborators have conducted *in vivo* experiments in the rabbit which leave little doubt that the platelets are the chief histamine source in this species.

Although the present studies are limited largely to the rabbit, a few experiments on dog blood have made possible the conclusion that in this species, contrary to the findings in the rabbit, the platelets contribute relatively little to the total blood histamine. Hence it would seem that there exists a qualitative as well as a quantitative difference in the histamine content and distribution in the blood of the species studied. Therefore, one must avoid applying too broadly conclusions based on blood histamine studies in single species.

On the basis of platelet volume and platelet histamine determinations, a fact of interest appears, namely, that rabbit platelets contain approximately 400 gamma of histamine per cubic centimeter. This is a value far in excess of the figures cited by various authors for other tissues. The possibility exists that the high histamine concentration in lung tissue, on the one hand, and in the platelets on the other, may be more than coincidental, since Howell and Donahue (14) have recently shown that the lungs are the principal source of these blood elements.

SUMMARY AND CONCLUSIONS

1. A modified method for the extraction of blood histamine by electrodialysis and assay on preserved isolated guinea-pig intestinal strips is described.

2. Quantitative studies have been made on the presence and distribution of histamine in blood of the rabbit and dog. Evidence is presented which supports the identity of histamine with the active substance in electrodialysates of rabbit blood.

3. The histamine activity of rabbit blood is located almost exclusively in the platelets. In dog blood, on the other hand, the platelets possess only a small share of the total blood histamine.

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BIOCHEMICAL CHARACTERISTICS OF DENERVATED SKELETAL MUSCLE, AT REST AND AFTER DIRECT STIMULATION

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The atrophy of muscle which occurs following injury to the anterior horn cells in poliomyelitis and which can be duplicated experimentally by section of the motor nerve connections, consists in the final analysis of a loss of muscle protein and its replacement by fibrous tissue and fat. From a dynamic standpoint this must result from either an increased proteolysis, a decreased protein synthesis or both. The disturbance in protein metabolism may be primary or secondary.

The present state of knowledge does not permit of a decision as to whether the muscle atrophy following denervation is due to a primary failure in protein metabolism, or whether the latter is secondary to a disturbance in the carbohydrate cycle. The second alternative offers the most feasible approach to the problem in view of the relatively advanced state of knowledge of the subject and the existence of more adequate methods of investigation. The relevant literature (up to 1939) has been collected and reviewed by Tower (1). Her survey shows that different phases of the biochemistry of denervated muscles have been studied by a number of workers in various ways. However, no study has appeared in which the many observations have been made on the same species of animal under the same conditions; nor has it been attempted to link the observed biochemical changes in a causative manner with the muscular atrophy which follows denervation. We wish to report such an attempt.

I. THE LEVELS OF CARBOHYDRATE AND PHOSPHATE COMPOUNDS IN NORMAL AND DENERVATED MUSCLE. *Methods.* The animals used were healthy adult rats of both sexes, whose weights ranged from 180 to 220 grams. The diet consisted of "Purina" Fox Chow supplemented with 10 per cent powdered skim-milk. Two animals were kept per cage during the experimental periods.

The muscles of the hind limbs were denervated by sectioning the sciatic and femoral nerves on one or both sides depending upon the type of experi-

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ment. The normal limb served as a control for the unilaterally denervated animals. Normal animals of the same weight and sex were used as controls for the bilaterally denervated rats. In all the experiments the gastrocnemius muscles were used for chemical analysis, at various intervals after the motor innervation had been severed. These intervals will be indicated in the text.

The various chemical determinations were done by the following methods:

1. *Inorganic and creatine phosphate:* Fiske and Subbarow (2) as adapted to the photoelectric colorimeter.

2. *Adenosine triphosphate:* Hydrolysis in N HCl at 100°C. for 7 minutes (Lohmann, 3).

3. *Hexosemonophosphate:* Formation of the water soluble barium salt (Needham, 4).

TABLE 1

SUBSTANCE	18 NORMAL RATS			20 BILATERALLY DENERVATED 14 DAYS			PER CENT DIFFERENCE
	Min.	Max.	Ave.	Min.	Max.	Ave.	
Glycogen	400	700	507	100	350	241	−52.5
Lactic acid	14	33	24	16	44	27	
Creatine phosphate	50	56	53	30	46	38	−28.3
Inorganic phosphate	22	34	26	17	31	21	
Adenosine triphosphate	31	41	37	18	33	25	−32.0
Hexosemonophosphate	9	18	14	10	19	16	
Hexosediphosphate	5	12	8	6	17	10	
Total acid soluble phosphate	152	207	194	131	190	176	

All values are in milligrams per 100 grams of muscle.

4. *Hexosediphosphate:* Hydrolysis in N HCl at 100°C. for 100 minutes (Lohmann, 3).

5. *Total acid soluble P:* Ashing with H_2SO_4 and H_2O_2 (Needham, 4).

6. *Glycogen:* Good, Kramer and Somogyi (5).

7. *Lactic acid:* Adaptation of micromethod of Miller and Muntz (6).

Results. The data are summarized in table 1. These results show a significant change in three substances. The glycogen concentration in the denervated muscle is less than half of the normal value. The creatine phosphate and adenosine triphosphate contents of denervated muscle are decreased by one-third as compared with normal control muscles.

Comment. Our results are in agreement with those of Hines and Knowlton, (7) (8) who found normal values for total acid soluble phosphate, low values for creatine phosphate and adenosine triphosphate and small changes in the inorganic phosphate content. Zanghi (9) also showed the

fall in creatine phosphate of denervated muscle and observed that it occurred after fibrillation has set in. There is agreement also as to the fall in glycogen content of paralyzed muscle (7).

This picture of the altered resting values of glycogen, creatine phosphate and adenosine triphosphate does not by itself throw any light on the dynamics of the glycolytic system. Are these quantitative changes associated with significant alterations in the enzyme systems operating in the glycolytic scheme? To answer this question we have investigated the ability of denervated muscle to phosphorylate carbohydrates and produce lactic acid from them.

II. PHOSPHORYLATION AND GLYCOLYSIS OF NORMAL AND DENERVATED MUSCLE IN VITRO. *Methods.* Muscle extracts were prepared according to the method of Kalckar (10) with M/30 Sorensen phosphate buffer. These extracts were incubated with the desired carbohydrate at 37.5°C.

TABLE 2

SUBSTRATE	NO. OF EXPERIMENTS	MGM. P ESTERIFIED IN 60 MINUTES					
		Normal limb			Denervated limb		
		Min.	Max.	Ave.	Min.	Max.	Ave.
Glycogen	6	0.33	0.60	0.51	0.18	0.39	0.30
Glucose	3	0	0	0	0	0	0
Fructose	3	0	0	0	0	0	0
Glycogen + glucose	4	0.24	0.36	0.30	0.17	0.22	0.19

for varying periods of time. NaF $\left(\frac{M}{200}\right)$ was used to inhibit dephosphorylation.

The in vitro production of lactic acid by the muscles was investigated by preparing a muscle brei and incubating it with glycogen under anaerobic conditions. A typical vessel contained: 1, 200 mgm. of muscle brei; 2, 25 mgm. of glycogen; 3, 2.5 cc. Hastings-Ringer solution buffered with M/15 phosphate buffer; 4, the gas phase was N_2.

Results. Table 2 shows that denervated muscle (14 days after nerve section) exhibits a lessened ability to phosphorylate glycogen. Neither the normal controls nor the paralyzed muscles are able to phosphorylate glucose or fructose. But both of these hexoses inhibit the phosphorylation of glycogen by muscle (table 2). The rate of anaerobic lactic acid production (table 3) lies in the same range in both normal and denervated muscles. The same holds for the rate of disappearance of glycogen (table 3).

Comment. It is apparent that the mechanism for the production of lactic acid from glycogen is not impaired despite the apparent lower

phosphorylosis of glycogen. Since this process involves all the enzyme systems known to be involved in glycolysis, no serious deficiency in any of these systems can exist.

The question then arises as to whether the difference between denervated and normal muscle in regard to the levels of glycogen, creatine phosphate and adenosine triphosphate, may not be due to a disturbance in the oxidative systems present in the tissue. Therefore we have studied the overall O_2 consumption, the succino-oxidase activity and the presence of other common dehydrogenase systems.

III. In vitro respiration of normal and denervated muscle. A. *Oxygen Consumption. Methods and results.* Respiration was measured in Barcroft-Warburg manometers. Temperature of the bath: 37.5°C. Gas phase: 100 per cent O_2. Medium: Hastings-Ringer's solution.

TABLE 3

NO. OF EXPERIMENTS	PER 1 GRAM OF MUSCLE	NORMAL LIMB		DENERVATED LIMB	
		30 min.	60 min.	30 min.	60 min.
6	Mgm. lactic acid formed	4.15	6.25	4.00	5.98
6	Mgm. glycogen broken down	6.10	17.25	6.55	14.85

TABLE 4

NO. OF EXPERIMENTS	DETERMINATION	PREPARATION	NORMAL LIMB	DENERVATED LIMB
6	Q_{O_2}	"Strips"	1.8–2.7	1.5–2.4
10	Q_{O_2}	Brei	3.8–6.7	2.1–4.5
	Q_{O_2} (Succ.)	Homogenized tissue	4.5–6.2	4.7–5.5

Our data in table 4 confirm the previous work by Knowlton and Hines (11) and E. Fischer (12) in showing that the in vitro O_2 consumption of denervated muscle differs little if at all from the normal. Because denervated muscle has a greater proportion of fibrous tissue than normal, and because oxygen consumption is expressed in terms of muscle weight, the above data suggest that the O_2 consumption of the active cells of the denervated muscle is actually increased.

Comment. The measurement of metabolic reactions in vitro does not give a quantitative picture of events in vivo. Although it is evident that the capacity of muscle tissue to use up O_2 is not impaired by denervation, this does not necessarily indicate that the O_2 is used normally. The total O_2 consumption of a tissue is the sum derived from all oxidative reactions occurring simultaneously. Despite normal values for Q_{O_2} the component reactions may be different. To obtain some information about this we

have determined the (1) succino oxidase activity and the (2) presence or absence of dehydrogenases for a number of metabolites.

B. *Succino-oxidase Activity. Methods and results.* The succino-oxidase activity was measured by the method of Elliott (13).

Table 4 shows that the succino-oxidase activity of denervated muscle exhibits a barely significant decrease as compared to normal muscle. Comment is reserved to the end of the next section.

C. *Dehydrogenases. Methods and results.* The presence of dehydrogenases for various substrates was tested by incubating muscle brei, suspended in Ringer's solution, in evacuated Thunberg-Keilin tubes. The main chamber contained 200 mgm. of muscle brei, 2 cc. of Ringer's solution and 0.2 cc. of M/1000 methylene blue; 0.2 cc. of a M/100 solution of the substrate to be tested was placed in the hollow stopper.

The metabolites examined in the above manner were as follows: glucose, lactic acid, pyruvate, fumarate, succinate, malate and citrate. There was no significant difference between the results obtained with denervated as compared to normal muscle.

Comment. Succino-oxidase activity provides some measure of the capacity of the cytochrome-cytochrome oxidase system. The activity of the dehydrogenases is involved in the first part of the oxidative chain.

Our data on succino-oxidase activity do not agree with those of Knowlton and Hines (11) who reported that the succino-oxidase activity is lowered about 50 per cent by denervation. However, their figures for the succino-oxidase were obtained with the methylene blue technique, so that it is hard to compare our results with theirs, as regards the normal or the denervated muscle. Our results indicate that denervation does not lead to either a quantitative or qualitative impairment of the oxidative capacity of the muscle fiber. The various dehydrogenase activities which we tested were present and the cytochrome-cytochrome oxidase system, though slightly decreased, was not impaired to a degree that would limit the oxygen consumption of the entire system. Thus the transfer of H_2 from substrate to molecular O_2 in paralyzed muscle proceeds through normal pathways despite the lack of nerve supply.

In view of the above it seemed logical to seek the explanation for the lowered levels of glycogen, creatine phosphate and adenosine triphosphate of denervated muscle, in terms of dynamic relationships which can be adequately studied only in vivo. For this purpose we undertook to examine the biochemical behavior of denervated muscle in the intact animal during work and recovery.

IV. THE METABOLISM OF NORMAL AND DENERVATED MUSCLE DURING WORK AND RECOVERY. *Methods.* For the investigation of the biochemical changes during contraction of the intact muscle in the living organism, we adapted the technique of Sacks (14). The gastrocnemius was dissected

free, under avertin anesthesia, without damage to its blood supply. It was then left at rest for 10 to 15 minutes, to allow for the restitution of any biochemical changes resulting from the trauma of the dissection. The muscle was then caused to work by stimulating it directly with a faradic current (induction coil) for 20 seconds. In a particular muscle the desired interval of recovery from the tetanic contraction was allowed (0, 10, 20, 30, 40 and 60 minutes). At the end of this period the muscle was quickly removed by severing its origin and insertion and immediately frozen between blocks of solid CO_2. The frozen material was weighed

TABLE 5

(72 animals; 408 determinations)

	DAYS AFTER DENERVATION	RESTING			IMMEDIATELY AFTER 20" TETANUS			RECOVERY PERIOD																	
								10 minutes			20 minutes			30 minutes			40 minutes			60 minutes					
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.			
Glycogen	0	400	700	507	200	500	333	340	350	340				260	430	345				420	460	440			
	1½-2	400	500	450	150	390	248	280	330	305	200	310	255				300	310	310	350	400	380			
	4½	170	420	268	50	230	124	50	140	108	120	170	140							110	180	145			
	14	100	350	241	70	330	150	190	190	190	160	160	160	200	200	200				170	170	170			
Lactic acid	0	14	33	24	102	102	102	38	38	38	49	49	49	22	22	22	23	23	23	23	24	24			
	1½-2	22	41	29	85	161	120	30	45	38	29	32	31				29	40	34	26	32	29			
	4½	17	39	29	78	130	100	19	59	44	29	73	53							25	38	32			
	14	16	44	27	43	88	63	46	46	46	30	30	30	31	31	31	18	19	19	32	33	32			
Inorganic phosphate	0	22	34	26	32	43	41	20	31	26	22	23	23	19	23	21	21	21	21	23	24	24			
	1½-2	17	31	23	29	58	43	26	26	26	24	30	27				22	27	24						
	4½	18	25	20	26	49	40	17	27	20	22	23	23							22	31	27			
	14	17	31	21	23	51	30	25	25	25	27	27	27	19	19	19	25	27	26	22	23	23			
Creatine phosphate	0	50	56	53	8	39	21	36	55	46	54	57	56	49	55	52	53	53	53	50	51	51			
	1½-2	52	64	56	14	44	27	52	56	54	48	53	51				41	48	45	50	53	51			
	4½	44	58	49	19	30	23	35	55	44	37	55	43							44	50	47			
	14	30	46	38	15	31	22	30	30	30	28	28	28	31	31	31	34	35	35	34	34	34			
Adenosine triphosphate	0	31	41	37	31	40	35	31	36	34	32	37	35	31	36	34	29	29	29	31	33	32			
	1½-2	34	40	37	31	39	35	39	40	40	38	40	39				33	35	34	36	38	37			
	4½	22	36	32	23	36	31	22	35	31	33	38	35							37	38	37			
	14	18	33	25	21	27	26	26	26	26	21	21	21	28	28	28	33	33	33	32	32	32			

and divided for various chemical determinations. The chemical determinations done included the following substances: glycogen, lactic acid, inorganic phosphate, creatine phosphate and adenosine triphosphate.

The comparison between the normal muscle and the paralyzed muscle, at the time when the latter might be expected to show the full effects of denervation, was made using normal rats and animals 14 days after bilateral denervation of the hind limbs. In order to observe the possible coincidence of chemical changes with the onset of fibrillation, which is known to start between 3 to 5 days after denervation, comparisons with the

normal were also made on rats which had been denervated for periods of 1½ to 2 and 4½ days respectively.

Results. The data are presented in table 5. The most significant changes are seen in connection with glycogen resynthesis. It is evident that glycogen breakdown and recovery is normal 1½ to 2 days after denervation, before fibrillation has begun. By 4½ days, which is shortly after the beginning of fibrillation, there is already a dramatic drop in the initial glycogen level and a pronounced impairment in the ability of the muscle to restore its glycogen. The longer interval which elapses between 4½ and 14 days makes very little further difference in glycogen breakdown and resynthesis.

The other marked difference between the normal and denervated muscle is in the breakdown and resynthesis of creatine phosphate. Unlike the changes in the glycogen these effects do not appear sharply at the onset of fibrillation, but seem to follow and perhaps are consequent to those of glycogen. Fourteen days after denervation the resting level of creatine phosphate is definitely lower than normal and the rate of its resynthesis is slow.

The lactic acid and inorganic phosphate show only minor changes. This is to be expected as regards readily diffusible substances which rapidly equilibrate with the blood.

Adenosine triphosphate is definitely decreased after 14 days of denervation. It should be noted, however, that this substance does not change significantly during contraction and recovery even of normal muscle, and hence its rôle in our comparison of dynamics may be discounted.

Discussion. Our results show that immediately after nerve section and for 3 to 4 days thereafter, the chemical constituents and reactions which we have investigated remain indistinguishable from the normal. This is true as regards the carbohydrate levels, the content of creatine phosphate, adenosine triphosphate, inorganic phosphate and lactic acid. Following stimulation of the muscle the changes in these compounds in the denervated muscle resemble those which occur in normal muscle both as regards direction and extent. This period corresponds to that during which no perceptible atrophy occurs and in which the muscle has been found to appear very nearly normal by gross and histological examination (1). But perhaps a more important known characteristic of this period is the fact that the muscle is truly quiescent for no filbrillation has yet begun. Thus in all respects it appears that the pathologic process which sooner or later follows motor nerve section is not the immediate result of the loss of the nervous connection but depends upon a secondary change which takes a number of days to appear.

The beginning of the period during which both anatomic and chemical changes appear seems to coincide with the onset of fibrillation (1, 15).

During this period the muscle content as regards glycogen, creatine phosphate and adenosine triphosphate decreases. The level of inorganic phosphate is normal or somewhat raised. The lactic acid content is normal. Following direct stimulation of the muscle the chemical changes which occur during tetanic contraction and recovery are qualitatively similar to those which occur in normal muscle, but quantitatively reduced.

Our further observations bearing on the possible reasons for the quantitative differences between normal and fibrillating denervated muscle do not reveal anything which could not be accounted for by the fibrillation. Thus the *in vitro* O_2 consumption per unit dry weight of denervated muscle (which has ceased fibrillating by the time measurements are made) compares to that of the normal. The succino-oxidase activity, which may be taken as a rough measure of the cytochrome-cytochrome oxidase system is somewhat reduced but would be adequate for even greater rates of oxygen consumption than those observed. Furthermore the various dehydrogenases which we tested by the Thunberg technique were found to be present and to yield activities comparable to those observed in normal muscle. There is therefore no reason to suppose that the oxidative mechanisms in the denervated muscle pursue pathways other than those established for normal muscle, and there is no evidence that the denervated muscle is different from the normal, once it has ceased fibrillating, except in so far as it may show the effects of previous prolonged work (low levels). In this connection it seems to us significant that many years ago Langley (16) observed that the *in vivo* O_2 consumption of actively fibrillating paralyzed cat muscle was greater than normal as judged by arterio-venous O_2 differences. In the light of our present work this indicates that in the intact organism the paralyzed muscle because of the continuous activity due to fibrillation, oxidizes its metabolites not only in a normal manner but at a rate greater than normal. An interesting analogy exists between such a muscle and the muscle exhibiting the increased metabolic activity consequent to hyperthyroidism. The hyperthyroid muscle has a low carbohydrate content and reduced levels of creatine phosphate (17, 18). It is also noteworthy that the same probable etiologic agent (fibrillatory muscle twitching) is also present, and that similar consequences, namely, loss of muscle substance and weakness, ensue.

On the basis of the above it is possible to construct the following working hypothesis. The severance of the motor innervation leads to a disorganized but increasing activity of the muscle fibres. This is followed by the depletion of the carbohydrate stores at a rate greater than can be restored by the blood transport mechanism and the synthetic processes in the muscle. The decreased amounts of intermediary metabolites resulting from a rate of catabolism which closely approaches the rate of supply, affords insufficient raw material for synthetic recovery processes. This

may very well apply to protein synthesis as well as to carbohydrate. In this connection it is known that the synthesis of the amino acids essential for complete protein structure necessitates the presence of keto acids, which result from carbohydrate breakdown and are necessary for the process of transamination (19).

It remains to be proven whether the chemical changes which accompany the fibrillation of the denervated muscle of the rat are indeed responsible for the muscular atrophy. While the present work was in progress Solandt and Magladery published an abstract (20) in which they reported that the administration of quinidine inhibited the fibrillation in the muscles of denervated rats without significantly influencing the rate of atrophy. Evaluation of their results must await the publication of their full data, but it is evident that the interpretation of their work may depend upon the manner in which quinidine abolishes fibrillation. We have done preliminary work on the influence of prostigmine and atropine respectively on the atrophy of denervated muscles. The actions of these materials are probably exerted on the physiological mechanism of fibrillation (acetylcholine (21, 22)). We have found that prostigmine, which increases fibrillation and atropine which does the reverse, respectively increase and decrease the rate of atrophy of the denervated gastrocnemius muscle of the rat.

SUMMARY AND CONCLUSIONS

1. The biochemical characteristics of denervated skeletal muscle, previously a subject of fragmentary reports, have been studied on the same species of animal (albino rat) and under the same experimental conditions. An attempt has been made to relate the observed biochemical changes to the muscular atrophy which follows denervation.

2. Fourteen days after denervation, at a time when significant atrophy has developed, the gastrocnemius muscle of the rat exhibits low levels of glycogen, creatine phosphate and adenosine triphosphate. There is no significant difference from the normal as regards the following: lactic acid, inorganic phosphate, hexose monophosphate, hexose diphosphate and total acid soluble phosphate.

3. After a similar period of denervation determinations of phosphorylation, glycolysis, in vitro O_2 consumption, succino-oxidase and dehydrogenase activities indicate that the transfer of H_2 from substrate to molecular O_2 in the denervated muscle proceeds through the normal pathways, and at a rate which is adequate for normal respiration.

4. An examination of the biochemical behavior of denervated muscle in the intact animal during work and recovery has revealed that glycogen breakdown and resynthesis is normal at $1\frac{1}{2}$ to 2 days after denervation, before fibrillation has begun. After the onset of fibrillation there is a

dramatic drop in the initial glycogen level and a pronounced impairment in the ability of the muscle to restore its glycogen after contraction. Similar but somewhat delayed effects have been observed as regards creatine phosphate.

5. Our observations do not reveal any biochemical changes in the denervated muscle which could not be accounted for by the onset and continuation of fibrillation. It is suggested that the atrophy of denervation is an "overwork" atrophy. Work in progress indicates that the atrophy can be increased or diminished by agents which cause an increase or decrease, respectively, in the degree of fibrillation.

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THE DISTRIBUTION OF RADIOACTIVE ISOTOPES OF IODINE IN NORMAL RABBITS[1]

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In this report we have surveyed the distribution of radioactive iodine in the tissues of the rabbit at various periods after intravenous injection in order that later experiments relating to the iodine metabolism may be more clearly defined.

The iodine content of various tissues of the rabbit has been studied using chemical methods (1). However, these procedures are not appropriate for studying the *rate of distribution* of iodine introduced into the rabbit (2). By the use of radioactive iodine the distribution rates may be obtained following the administration of small doses of iodine (3, 4). These values establish the normal ranges of distribution rates for a given tissue as well as the variations which normally occur in the distribution rates to different tissues. Clinical use of this method has already been of some value in certain types of human thyroid disease (3). However, once normal values for iodine distribution in a suitable experimental animal are determined, the techniques of experimental pathology can be applied to expedite the study of variations in iodine metabolism characteristic of disease.

Radioactive iodine is prepared by bombarding metallic tellurium or one of its compounds in the cyclotron with six and one-half million electron volt protons. The nuclear reactions may be written as follows:

$$\mathrm{Te^{128} + H^1 \longrightarrow I^{128} + n^1}$$
$$\downarrow \quad \text{25 minutes isotope}$$
$$\mathrm{Xe^{128} + e^-}$$

$$\mathrm{Te^{130} + H^1 \longrightarrow I^{130} + n^1}$$
$$\downarrow \quad \text{12.5 hours isotope}$$
$$\mathrm{Xe^{130} + e^-}$$

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Two radioactive isotopes of iodine with half-lives of 25 minutes and 12.5 hours, respectively, are thus formed. Isotopes with half-lives of longer duration are also produced but the isotope with a half-life of 12.5 hours predominates in the samples used.

Preparation of radioactive iodine for injection. Fifteen milliliters of concentrated C. P. nitric acid were placed in a 25 x 200 mm. test tube and the platinum foil bearing the tellurium and radioactive iodine dropped in along with about 5 mgm. or less of crystalline iodine. A stopper bearing a glass delivery tube was inserted and the delivery tube immersed in a narrow column containing carbon tetrachloride to absorb any iodine driven off. The nitric acid was heated nearly to boiling, poured into a small separatory funnel, and the iodine repeatedly extracted with ten milliliter portions of carbon tetrachloride until the acid layer was free from radioactive material as tested by placing the funnel near the ionization chamber of the counter. Another portion of the concentrated nitric acid was added to the foil and tellurium, brought to boiling temperature and the boiling continued until the tellurium dissolved. The subsequent extraction was repeated. The carbon tetrachloride fractions were united and extracted repeatedly with distilled water containing a few drops of a saturated sodium sulfite solution. The carbon tetrachloride fraction was discarded when it no longer contained any radioactive material. The excess sulfite was oxidized to sulfate with a few drops of dilute solution of potassium permanganate. The solution containing the radioactive iodine was evaporated to a volume of 25 ml., a 1 ml. aliquot removed and made up to 10 ml. for a radioactive standard from which the composite decay curve of the mixed iodine isotopes was determined.

Experimental procedure. Normal, young, adult stock, New Zealand, white rabbits were used throughout these experiments. These rabbits were all from the same stock, weighing 2.5 to 3.0 kgm., and were approximately of the same age (5–6 mos.). They were kept separately in wire-bottom cages and fed oats and dry alfalfa for 2 to 3 weeks before use, and maintained on this diet after the injection of radioactive iodine. The city water contains approximately 5 parts of iodine per billion, and no iodine was added to it.

Ten milliliters of the iodine-bearing solution were injected slowly into an ear vein of a rabbit. No untoward reactions occurred. At intervals the animals were sacrificed by injecting air into an ear vein, and samples of various tissues taken. The weight of each entire organ was determined and a weighed aliquot taken for examination. The sample was minced and dissolved in dilute NaOH with heat. The solution was then diluted to 10 ml., and a 2 ml. aliquot portion placed in the cup of the ionization chamber of the Geiger-Muller scale-of-four counter (5) for a determination of the radioactivity. The urine volume was determined and a 2 ml.

aliquot counted. For blood measurements, 2 ml. of serum were used in each instance. The counting procedure has been described in detail elsewhere (5).

In a series of experiments, aliquots of radioactive iodine having a known activity were dissolved in 10 per cent NaOH in some instances and in others placed in the NaOH solution in which approximately 1 gram of organic material had been ashed. The counts in both instances were identical, demonstrating that there is no appreciable absorption of beta rays by the ash of the organic material.

DATA. Table 1 gives a detailed statement of the various determinations. In the first column at the left are listed the individual tissues studied, for example, thyroid, blood, urine, etc. In each of the other columns the per cent of the original dose of radioactive iodine per gram of the various tissues is recorded. Each vertical column represents the results on one rabbit. The tissues were taken at the times indicated following the intravenous administration of radioactive iodine.

TABLE 1

Distribution of radioactive iodine in rabbit tissues at various times after injection (per cent dose per gram wet tissue)

	5 MINUTES	5 MINUTES	35 MINUTES	1 HOUR	1 HOUR	2 HOURS	2 HOURS	4 HOURS	8 HOURS	9 HOURS	12 HOURS	19 HOURS	24 HOURS	48 HOURS
Thyroid	1.2		4.1	8.2	0.75	16	23	0.97		1.43	2.4	3.2	2.4	15
Blood	0.16	0.12	0.10	0.01	0.09	0.08	0.06	0.11	0.11	0.05	0.08	0.003	0.02	0.06
Urine	0.19		0.26	0.03		0.04	0.06	0.02	0.05	0.31	0.12	0.11	0.08	0.06
Liver	0.01		0.04	0.11		0.09	0.02	0.06	0.08		0.009	0.01	0.003	0.02
Lung	0.15	0.005	0.09	0.10	0.14	0.08	0.05	0.09	0.34		0.009	0+	0.01	0.04
Kidney	0.05	0.12	0.11	0.09		0.12	0.12	0.09	0.25		0.01	0.009	0.02	0.09
Spleen	0.03		0.04	0.10		0.08	0.01	0.03			0.008	0	0	0.02
Submax	0.02	0.07	0.08	0.20		0.06	0.03				0.007		0.007	0.04
Testicles	0.005		0.03			0.008						0	0.008	0.02
Adrenals			0.07								0.002	0	0	0
Muscle	0.003		0.04				0.004				0.003	0.03	0.002	0.01
Heart	0.01	0.06	0.05				0.05				0.007	0	0.004	0.03
Bile	0.51	0.02	0.17								0.03	0.004		0.01
Diaphragm			0.04									0		0.02
Feces												0		0.06
Skin-fur*		0.03	0.10				0.004				0.009	0	0.006	0.04
Fur*		0.02	0.04								0	0	0.01	0.09

* Probable contamination.

Blood. The radioactive iodine is injected directly into the ear vein, yet at the end of 5 minutes, approximately 70 per cent of the injected material has been removed from the blood stream. The blood radioiodine level falls during the 48 hours studied. However, there is a wide variation, e.g., for period over 12 hours, three determinations gave from 0.003 to 0.06 per cent of the original dose present per cubic centimeter of serum.

Urine. Radioactive iodine from the blood appears almost immediately in the urine. Two animals were sacrificed 5 minutes following the injection of the iodine. In one, none had reached the bladder urine but it had reached the kidney to the extent of 0.12 per cent of the injected dose per gram. In the other, the kidney contained 0.05 per cent per gram of the injected dose and the bladder urine 0.19 per cent per cubic centimeter. As much as 37 per cent of the injected dose may be excreted in urine in 9 hours.

Thyroid. The thyroid gland picks up a remarkable proportion of the dose. Five minutes following injection, the thyroid contains 1.2 per cent of the injected iodine per gram of thyroid tissue. Later the amount of radio-iodine in the thyroid increases (as much as 20 to 30 per cent of the dose). There is a considerable and unexplained variation in the percentage of the dose found in the thyroid.

Other tissues. In the other tissues examined, there was some indication of regularity in the percentage of the radio-iodine dose found per gram of moist tissue. Thus, lung and kidney usually contained relatively high percentages; liver, spleen, heart, bile, and submaxillary gland usually contained intermediate percentages; and muscle, skin, diaphragm and testicle contained low percentages of the dose. The low percentages reported for fur are probably the result of accidental contamination.

Discussion. The high content of the marked or radioactive iodine found in the thyroid may be a function of two factors: 1, increase in iodine content of the tissue; and 2, an increase of radioactive iodine by exchange because of the high "radioactive iodine to non-radioactive iodine" ratio in the circulatory system as compared to the low "radioactive iodine to non-radioactive iodine" ratio in the thyroid immediately after injection. Hertz et al. (4) have observed that markedly hyperplastic thyroid glands concentrate approximately 0.6 per cent of the injected material in about 40 minutes, and that normal thyroids only concentrate in the vicinity of 0.1 per cent of the injected amounts in 10 to 50 minutes. In experiments reported here the normal thyroid concentrates various amounts, fluctuating between 0.29 to 1.23 per cent of the dose given within the first hour, which, in general, confirms the results of Hertz et al. for normal tissues.

SUMMARY

1. Following intravenous injection of radioactive iodine, there is an immediate distribution to the various tissues from the blood with considerable fluctuation in the relative amounts found in any organ at the various periods observed.

2. The thyroid takes up a relatively large percentage of the injected dose.

3. The radio iodine appears promptly in the urine. Up to 30 per cent of the dose may be excreted in 9 hours.

4. Lung and kidney tissue contain relatively high percentages of the radio iodine dose; liver, spleen, heart, bile and submaxillary gland contain intermediate amounts; and muscle, skin, diaphragm, and testicle have low percentages.

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THE MAGNITUDE AND TIME OF DEVELOPMENT OF THE COLLATERAL CIRCULATION IN OCCLUDED FEMORAL, CAROTID AND CORONARY ARTERIES[1]

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Although anatomical studies (1, 2) reveal that collateral connections establish themselves in various body regions following ligation of the main artery to that region, we have only a limited knowledge concerning the magnitude, time of development and pressure relations of such collaterals. Accordingly, experiments were designed to study these phenomena following acute and chronic ligation of femoral, carotid and coronary arteries.

METHODS. 1. *Femoral and carotid artery ligation.* In acute experiments dogs were anesthetized with sodium pentobarbital, chloralose, or morphine and ether, and the blood rendered non-coagulable with heparin and chlorazol fast pink or pontamine fast pink. The femoral artery was ligated in the upper third of the thigh and the carotid artery at the level of the thyroid cartilage. A cannula was placed in the main artery or in a side branch peripheral to the point of ligation. Retrograde flows were measured in a graduate or with a volume recorder, and retrograde pressures, together with aortic or femoral pressures were determined with Gregg (3) manometers. Measurements were made immediately and frequently during several hours after ligation. In some experiments blood pressure was altered with an aortic clamp, neosynephrine or augmented venous return. In other experiments, following an increase in retrograde pressure and flow, the ligature was removed and re-applied later. In chronic experiments dogs were operated aseptically initially and retrograde flows and pressures determined. At intervals varying from one day to many months the retrograde flows and pressures were again measured. In some cases the vein draining the region fed by the artery was also ligated, while in others the potential collateral sources were investigated.

2. *Coronary artery ligation.* Measurements of pressure and flow were

[1] The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

[2] Hanna Fellow in Medicine.

[3] Rees Fellow in Medicine.

made in the same and in different dogs in a manner similar to that described for the femoral and carotid arteries, and for periods extending from a few seconds to seven days after ligation.

TABLE 1

EXP. NO.	DOG WEIGHT	DURATION OF OCCLUSION	BLOOD PRESSURE	RETROGRADE PRESSURE	FLOW	REMARKS
			Femoral (9 experiments)			
	kgm.				*cc./min.*	
1a	13	15 seconds	186/135 (femoral)	20/20	10	
1b	13	90 minutes	195/138 (femoral)	98/93	50	
2	12	5 days	140/108 (femoral)	82/74	100	
3	14	250 days	212/165 (femoral)	160/125	200	
			Carotid (8 experiments)			
4	13	1 minute	96/82 (carotid)	66/62	16	
5		3 hours	103/96 (carotid)		110	
			Coronary (25 experiments)—left descendens			
7a	18	2 minutes	65 (mean carotid)		2.4	
7b	18	20 minutes	100 (mean carotid)		5.8	
7c	18	41 minutes	65 (mean carotid)		2.8	
7d	18	55 minutes	65 (mean carotid)		3.2	
8a	12	35 minutes	118/94 (carotid)	31/10	1.9	
8b	12	6 hours	98/74 (carotid)	31/7	2.5	
9a	19	13 minutes	123 (carotid)		3.4	
9b	19	48 hours	85 (mean carotid)		6.8	
10a	15	7 days	102/74 (carotid)	68/22	8.5	
10b	15	7 days	121/91 (carotid)	101/65	18	Aortic clamp
10c	15	7 days	132/106 (carotid)	104/72	24	Aortic clamp
11a	20	15 seconds	107/86 (carotid)		2	
11b	20	30 seconds	97/75 (carotid)		0.7	Circumflex clamped
11c	20	45 seconds	83/60 (carotid)		1.1	Circumflex released
			Left circumflex			
12a	13.5	6 days	134/106 (carotid)	100/54	9.4	Aortic clamp
12b	13.5	45 min. later	95 (mean carotid)		6.0	Control
12c	13.5	47 min. later	95 (mean carotid)		4.0	Left descendens and vein ligated

RESULTS. *Acute experiments.* Typical retrograde flows and pressures from carotid, femoral and coronary arteries at periods varying from one minute to six hours following ligation are shown in table 1. Immediately

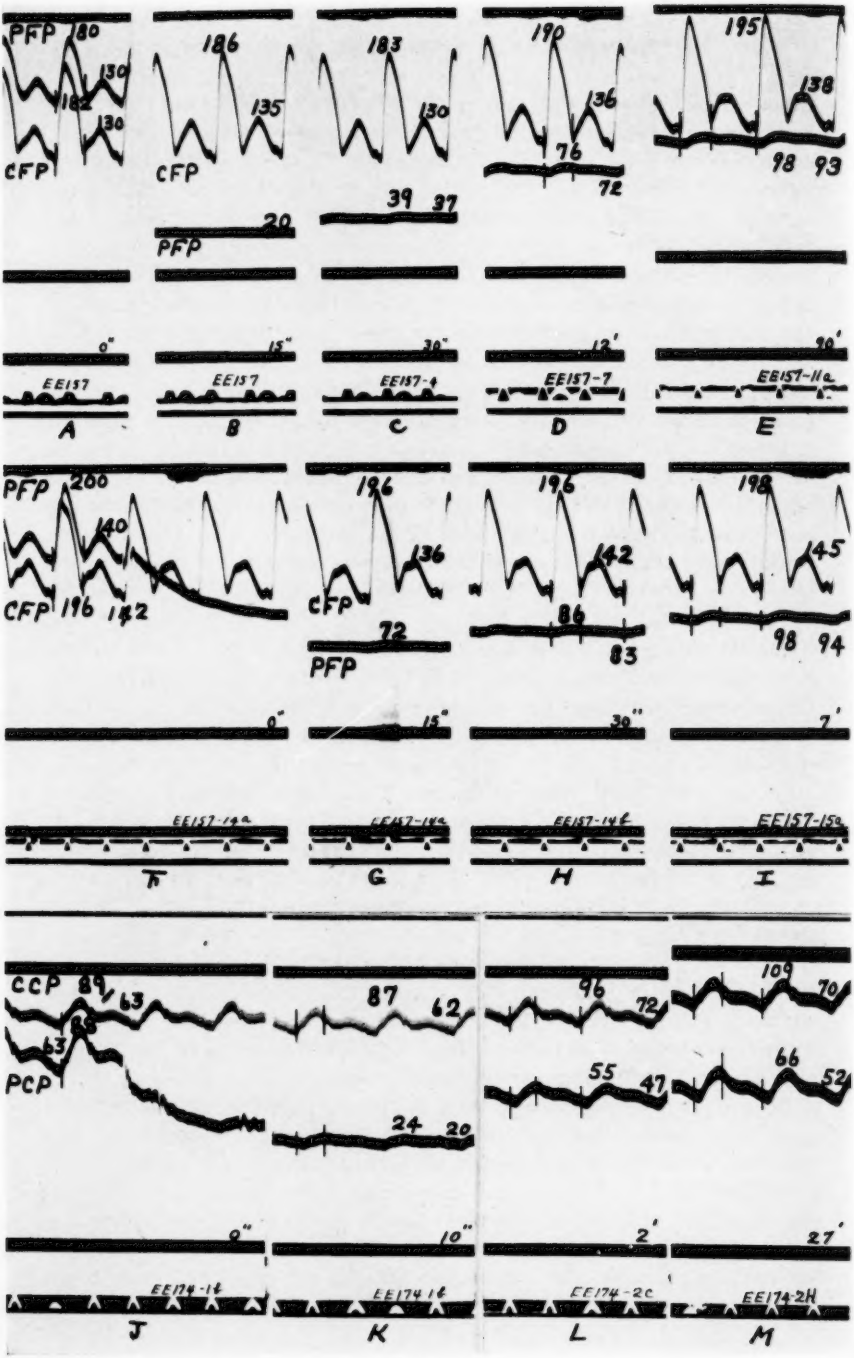
after occlusion these values are small. However, within a minute carotid and femoral retrograde pressures and flows increase and continue to increase for hours (cf. expt. 1). In figure 1 (A and B), within 15 seconds following femoral ligation, the peripheral pressure drops from the control femoral pressure of 180/130 mm. Hg to 20/20 mm. Hg and the pulse disappears. However, within 30 seconds in C a definite pulse is present and the pressure is 39/37 mm. Hg. In subsequent records the pressure and pulse continue to rise, rapidly at first in D (after 12 minutes' occlusion) and then more slowly until after 90 minutes (E) the pressure is 98/93 mm. Hg. Such a pulse bears no resemblance to the central femoral pulse and its rise follows the latter by 0.03 second. With such an increase in retrograde pulse and pressure the retrograde flow rises from 10 cc. per minute in B to 50 cc. per minute in E. Once open, such collaterals tend to remain patent for in F the femoral ligature was removed for one hour. In G the ligature is replaced and the retrograde pressure drops only to 72 mm. Hg in contrast to the 20 mm. Hg following the first occlusion. Furthermore, a small pulse is present, and within 7 minutes the pressure of 98/94 mm. Hg in I is equal to that (98/92 mm. Hg) present 90 minutes after the initial occlusion.

Similar changes in retrograde pressure and flow occur following acute carotid artery occlusion, figure 1, (J, K, L, M) with the following differences: 1, the carotid peripheral pressure always has a sizable pulse; 2, its limit of rise is reached more quickly, and 3, the pulse is patterned after the central carotid pulse.

On the other hand, contrasting with the rapid collateral development in the femoral and carotid arteries, is the small initial and slow increase in collateral function after coronary artery occlusion. (See table 1 and fig. 2.) In measurements on 17 dogs in which the mean blood pressure varied from 60 to 117 mm. Hg and the weight from 7 to 27 kgm., the flow varied from 0.5 to 5.8 cc. per minute, with only 4 of the values below 1 cc. per minute. With an average mean blood pressure of 84 mm. Hg, an average weight of 14.6 kgm., the average retrograde flow is 2.2 cc. per minute. The high flow is 5.8 cc. per minute with a weight of 18 kgm. and a blood pressure of 103 mm. Hg. The low flow is 0.5 cc. per minute with a weight of 10.5 kgm. and a blood pressure of 112 mm. Hg.

As evident in table 1, experiment 7, these values may slowly increase in any one heart during the first hour after occlusion. Figure 2 (A) was taken a few minutes after occlusion of the left descendens. The chest was then closed and normal respiration restored for 6 hours, at which time (fig. 2, B), in spite of considerable fall in aortic blood pressure and a constant peripheral coronary pressure, the flow increased slightly from 1.9 to 2.5 cc. per minute.

Chronic experiments. Typical results of retrograde flow and pressure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130
182
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98 93
0"
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A
15"
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B
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C
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D
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EE157-11a
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PFP 200
140
CFP 196 142
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136
CFP
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145
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0"
15"
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EE157-14a
EE157-14c
EE157-14f
EE157-15a
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CCP 89
63
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24 20
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72
55 47
109
70
66
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0"
10"
2'
27'
EE174-1f
EE174-1f
EE174-2c
EE174-2H
J
K
L
M

Fig. 1

are presented in table 1, and figures 2 and 3. After a few days of femoral and carotid artery occlusion the retrograde pulse and flow are large, but not much greater than after a few hours. However, after long-sustained occlusion the femoral retrograde pressure and flow become very large and the contour of the pulse resembles that of the central femoral pulse. In figure 3 A, after 250 days of occlusion, the retrograde pulse is 160/125 mm. Hg and the flow is 200 cc. per minute. Similar changes occur in the chronically occluded carotid artery (fig. 3, B, occluded 152 days). In both pulses, however, a considerable time lag is still present.

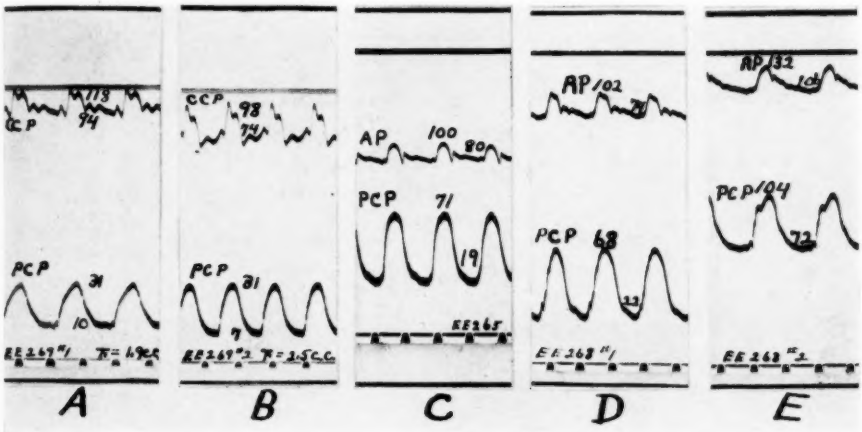

Fig. 2. Records showing changes in peripheral coronary pressure at different times after coronary occlusion. A, immediately after occlusion of the descendens. B, 6 hours later in the same dog. C, 48 hours after occlusion of descendens. D, 7 days after occlusion of the descendens. E, same, except aorta clamped. *PCP*, peripheral coronary pressure. *CCP*, central carotid pressure. Time, $\frac{1}{5}$ second.

Within 48 hours the coronary collaterals are also further extended. For example, in one dog in experiment 9 of table 1 the retrograde left descendens coronary artery flow increases, after 48 hours of occlusion, from the initial value of 3.4 cc. per minute at a mean blood pressure of 123 mm. Hg to 6.8 cc. per minute at a mean blood pressure of 85 mm. Hg. See

Fig. 1. Records showing development of retrograde pressure in acutely occluded femoral and carotid arteries. A, control for B, C, D and E, taken at varying times up to 90 minutes after femoral ligation. F, record taken one hour after femoral release in E and serving as control for femoral re-ligation in G, H, I. J, control for records K, L, M taken at varying times after carotid ligation. *PFP*, peripheral femoral pressure. *CFP*, central femoral pressure. *PCP*, peripheral carotid pressure. *CCP*, central carotid pressure. Time, $\frac{1}{5}$ second.

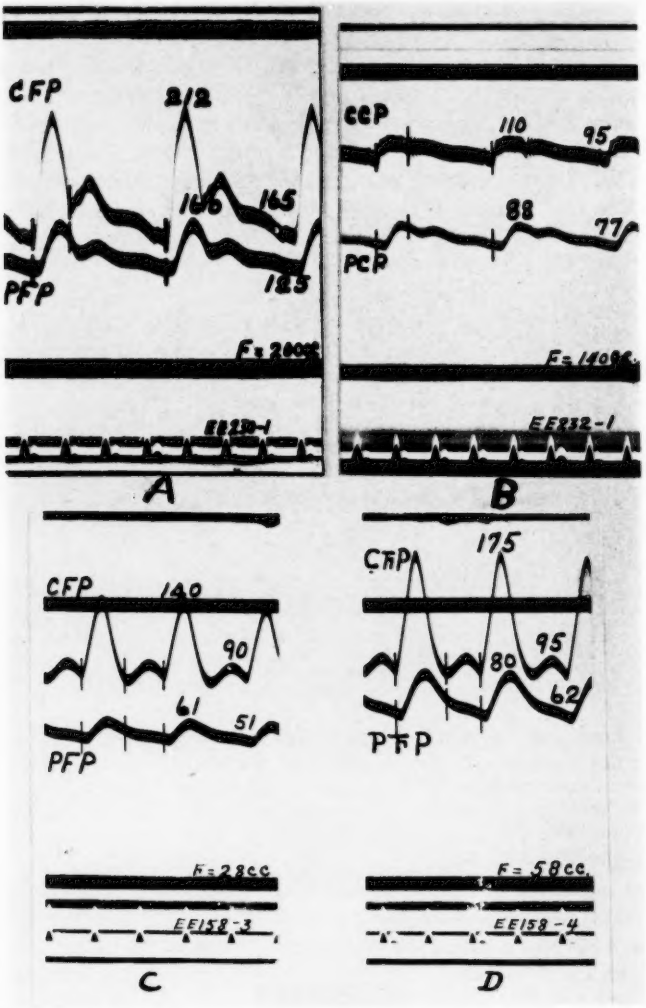

Fig. 3. Records illustrating high peripheral pulses after long continued carotid and femoral occlusion. A, after femoral occlusion for 250 days. B, carotid occlusion for 152 days. C, femoral occlusion for 90 minutes, and also serving as control for D in which venous return was increased. Letters and time same as in figure 1.

figure 2 C for records of left descendens peripheral coronary artery pressure after 2 days. These collaterals are still further augmented after 1 week, for, in table 1, experiment 10 (b) the left descendens artery has

been ligated for 7 days and the flow of 18 cc. per minute, with a mean blood pressure of 105 mm. Hg is greatly augmented over the highest acute value obtained in 17 dogs of 5.8 cc. per minute with a mean blood pressure of 103 mm. Hg. Such an early increase in collateral function has also been demonstrated for the circumflex artery (table 1, expt. 12). In spite of a

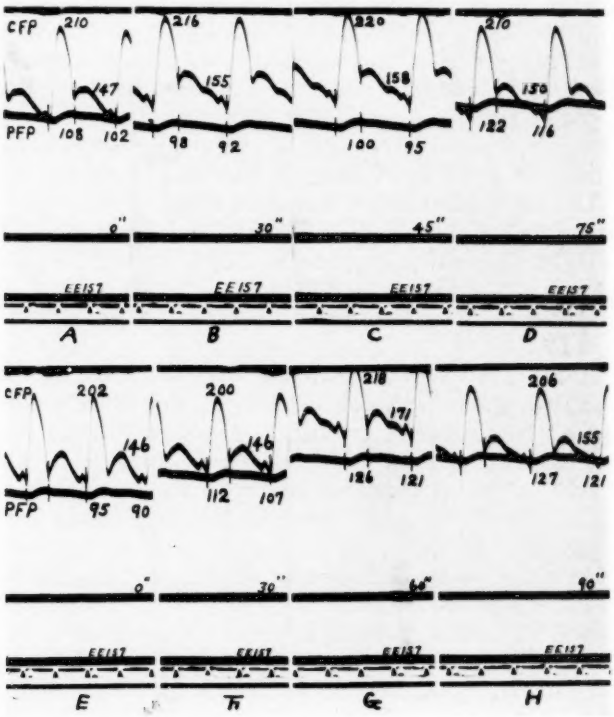

Fig. 4. Records showing dual effects of neosynephrine injection on the retrograde pressure in the acutely occluded femoral artery. A, control for B, C, D in which neosynephrine reduces the peripheral pressure. E, control for F, G, H in which neosynephrine increases the retrograde pressure.

rise in retrograde pressure and flow most of the myocardial area normally fed by an occluded coronary artery does not contract. However, within a minimum of 7 weeks such ischemic areas do contract and values for retrograde pressure and flow are still further augmented (4).

Responses of collaterals to different circulatory conditions. The effects of venous ligation, increased venous return, augmented blood pressure and neosynephrine on retrograde pressure and flow were studied.

Following ligation of both the femoral artery and vein in these experiments there is an increase in retrograde pressure and flow (record not shown). Similar results were found by other investigators (2). Increased venous return and elevated blood pressure likewise augment both the retrograde pressure and flow in the acutely and chronically occluded femoral and carotid arteries. In figure 3 C the femoral artery has been ligated for 90 minutes and the peripheral pulse is constant. Following an infusion of Locke's solution the retrograde pressure rises from 61/51 to 82/62 mm. Hg and the retrograde flow increases from 28 to 58 cc. per minute. Although neosynephrine usually increases both the retrograde pressure and flow, figure 4 shows that it may have a dual effect. The upper curves show an initial decrease of retrograde pressure in spite of a slight increase in aortic blood pressure, following intravenous neosynephrine. The lower curves show the usual effect. Following coronary sinus ligation both the retrograde coronary artery pressure and flow are increased (3, 5). Likewise, augmented venous return and increased blood pressure increase the retrograde coronary artery pressure and flow in the acutely and chronically occluded artery. (See fig. 2 (D and E) and table 1, expts. 10 and 7.)

Finally, the source of the retrograde flow was investigated by measuring the resulting changes in such flow following temporary occlusion of potential sources. As indicated in table 1, experiment 11, the retrograde flow from the acutely occluded left descendens artery decreased markedly following occlusion of the circumflex artery. After the circumflex artery was again released the retrograde flow increased in spite of a further fall in blood pressure. Similarly, the retrograde flow from the chronically occluded circumflex artery decreased by 33 per cent following occlusion of the left descendens artery (expt. 12).

Discussion. The general trend of collateral extension in the three typical arteries is summarized in schematic form in figure 5. In the occluded femoral artery the pulse disappears, the flow drops to a very low value, to be replaced within a few seconds by a rapidly rising pulse and flow during a period of hours, and by a more slowly rising pulse and flow during days and weeks. The carotid artery behaves similarly except that a pulse is never lost, the retrograde pressure and flow do not sink to such low levels and more quickly approach their original value and contour. In the coronary artery, however, the retrograde flow is at first small and remains so for hours; the pulse may be smaller or larger than before occlusion, but is always at a lower diastolic level. The diastolic flow and pressure gradually rise to sizable values within a few days (2–6); the pulse with the same or different contour may have the same, smaller or greater magnitude.

The above indicates that the rate of collateral development in various

body regions is influenced to a different extent by various factors. The mechanisms for the collateral reactions in figure 5 are 1, the opening of preëxisting but non-functioning collaterals by a combination of differential pressure, metabolites, and nerve action, and 2, the formation of new collaterals.

In the peripheral femoral and carotid arteries the existence of the initial rapid rise of the peripheral pressure and flow immediately after occlusion is evidence that the collaterals involved at this stage preëxist. The main initial impetus to their opening is presumably the augmented differential pressure, since 1, the collaterals function within a few seconds after occlu-

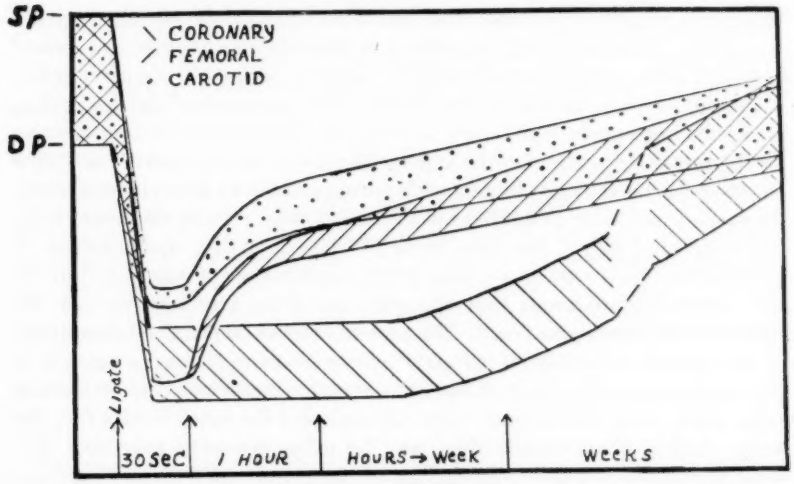

Fig. 5. Schematic drawing indicating the degree of collateral extension in the carotid, femoral and coronary arteries as evidenced by the retrograde flow, diastolic pressure and pulse. *SP*, systolic pressure. *D.P.*, diastolic pressure and flow.

sion; 2, the differential pressures are extremely high (166 and 63 mm. Hg respectively in fig. 1, B and K), and 3, procedures which increase further mechanically the differential pressure such as femoral vein ligation and elevation of systemic blood pressure (in the case of the carotid) give marked elevation of the peripheral pressure and flow.

The reason for the failure of the peripheral flow in an occluded coronary artery to increase for a number of hours and then only slowly in contradistinction to the femoral and carotid arteries is not known. Apparently the differential pressure is adequate and may approximate that in the carotid and femoral, while that in the right coronary may be even higher. For example, in figure 2 A the differential pressure averaged about 90 mm.

Hg, and yet after 35 minutes the retrograde flow was only 1.9 cc. per minute.

Following coronary artery occlusion the peripheral coronary diastolic pressure rises within 2 to 6 days and may, as in the case of the carotid and femoral arteries, be regarded as definite evidence of collateral extension. However, in contradistinction to the femoral and carotid arteries, the sizable pulse which persists after occlusion may show little or no increase as collaterals open. In other words, the pulse pressure here is not necessarily an index of collateral extension, but rather the resultant of various factors tending to make the pulse larger or smaller. The increased functioning of many small, deeply embedded coronary collaterals promotes the diastolic filling of the coronary bed and thus increases the systolic pressure and pulse. However, large superficial collaterals may acquire the central coronary pulse which is usually smaller than the peripheral coronary pulse.

Previous reports indicated that shortly after occlusion of the descending artery the retrograde flow ranges from 0.5 cc. (4) to 1.5 cc. (6) per minute. In the present experiments the retrograde flow from the acutely occluded left descendens artery is larger, with a range of 0.5 to 5.8 cc. per minute. The use of morphine and ether for anesthesia may explain the larger back flows reported here. Previous work (7) based on the small decline in peripheral coronary artery pressure following clamping of potential sources, was interpreted to mean that the other coronary arteries were not the source of the retrograde flow. The present studies confirm the magnitude of the decline of peripheral coronary artery pressure following ligation of potential sources of collateral flow, but actual measurements of retrograde flows show that, like coronary arteries occluded for many weeks (4), the major source of retrograde flow may be other coronary arteries. The most probable explanation for failure of the peripheral coronary pressure to decrease is that after occlusion the coronary bed is so poorly filled during diastole that further reduction in diastolic filling by ligating a coronary collateral is insufficient to affect the diastolic pressure and hence the pulse pressure.

SUMMARY

The time rate of collateral development has been studied in the femoral, carotid and coronary arteries, by means of the retrograde pressure and flow.

In the femoral artery immediately after occlusion the retrograde pressure and flow approximate 20 mm. Hg and 10 to 15 cc. per minute, respectively. Inmediately, a small pulse appears and these values rise rapidly for a few hours and then more slowly for days and weeks until pulse and flow may approach those existing in the other intact femoral artery.

In the carotid artery the initial retrograde pressure and flow are somewhat greater than in the femoral artery; a pulse is always present and these approach more quickly the normal for the other carotid.

Following coronary occlusion the retrograde flow is nearly constant at values between 0.5 to 5.8 cc. per minute and then after some hours it, together with the peripheral diastolic pressure, increases very slowly to obtain sizable values in a week or so. As in coronary arteries occluded for many weeks, the major source of such retrograde flow may be the other coronary arteries.

Evidence is given to indicate that initial retrograde flow is due to increased differential pressure opening preëxisting collaterals. The mechanism for further collateral extension is not known.

In addition to the flow, the rise of the peripheral diastolic pressure in all the arteries and the peripheral pulse in femoral and carotid may serve as an index of collateral extension.

The peripheral pulse and retrograde flow are elevated following increased venous return and augmented blood pressure, but may be either increased or decreased by neosynephrine.

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THE PATTERN OF SERUM PROTEINS DURING ACCELERATED GROWTH[1]

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Pursuing the general program of study of the chemical changes incident to growth, Smith and Gross (1937) have described changes occurring in the blood of albino rats following retarded growth as well as during unusually rapid development. It was demonstrated that animals in which increase in body weight had been restricted through lack of either the essential amino acid, lysine or of food energy, show an unusually rapid maturation of the blood picture as judged by the change in number of erythrocytes, concentration of hemoglobin and change in proportion of reticulocytes. Furthermore, upon realimentation either with a qualitatively adequate diet or with one providing requisite energy, a more youthful blood picture again appears and the process of hematologic maturation is resumed paralleling the accelerated somatic development. Smith and Gross ascribed the early rapid maturation of the blood to the stabilization of the blood volume induced by restriction of growth; the return to the more youthful blood picture upon realimentation seemed to parallel the increasing demand for blood which results when growth is resumed. The authors suggested that the rapidly expanding vascular bed may be more readily filled with fluid than with formed elements so that the immaturity of the blood picture following realimentation is the result of dilution accompanying or preceding resumption of hematopoiesis. The present study was undertaken in order to determine the chemical nature of this diluting fluid (plasma) during restriction of growth and during accelerated growth following realimentation.

PROCEDURES. The experimental conditions established by Smith and Gross (1937) were reproduced as closely as possible and hemoglobin determinations and erythrocyte counts were used to confirm the earlier observations. The pre-experimental diet of all animals was somewhat

[1] The data discussed in this paper were taken from a thesis submitted by E. S. Zawadzki in candidacy for the degree of Master of Science, Wayne University, 1940.

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richer in protein, however, than in the earlier study (see table 1), and the period of restricted feeding was shortened from six to three weeks. The data of Smith and Gross suggested this economy and it was shown by preliminary experiments that the results were not affected by this change in procedure. It was also demonstrated that the most profound change in the blood picture occurs three days after realimentation of the animals previously maintained on the restricted diets.

Male albino rats weighing 45 ± 5 grams at weaning (21 days of age) were given an adequate diet (pre-experimental diet, table 1) and 200 mgm. of yeast daily; those animals reaching 110 ± 5 grams body weight in 15 ± 2 days were changed to the experimental rations described in table 1.

TABLE 1

Composition of diets

	PRE-EXPERIMENTAL DIET	EXPERIMENTAL DIETS			
		Diet I	Diet II	Diet III	
Lactalbumin*	25	18		36	The vitamin B complex was supplied as dried yeast‖ apart from the experimental ration. The amounts given are noted in the text.
Gliadin**			18		
Dextrin†	44	51	51	23	
Hydrogenated fat‡	22	22	22	23	
Cod liver oil	5	5	5	10	
Salts§	4	4	4	8	

* Labco no. 15-42, Borden Company, New York; moisture 6.5 per cent, total nitrogen 11.2 per cent, ash 1.6 per cent.

** Made from wheat gluten; moisture 5.0 per cent, total nitrogen 13.3 per cent, ash 0.5 per cent.

† White commercial.

‡ Crisco.

§ Osborne and Mendel (1917).

‖ Northwestern Yeast Company.

They were so distributed as to place litter mates into each of the three experimental groups as far as possible. The animals were housed in individual cages and body weight and food intake for each animal were recorded. Group I, consisting of twenty animals, was allowed to grow normally, being fed an adequate diet. Nine animals, serving as weight controls, were bled at 36 ± 2 days of age and 110 ± 5 grams body weight and the serums obtained were analyzed; the rest of the animals, serving as age controls, were fed diet I ad libitum until killed at an age corresponding to that at which their mates in group II and III were killed. Group II received diet II ad libitum; ten animals were killed and analyses performed at the end of three weeks on this ration and ten were realimented for three days with diet I following the three weeks of diet II. Seventeen animals in group III served as calorie controls receiving diet III in such

quantity daily as to maintain their weight at the level of those in group II. Eight animals were used for determinations at the end of three weeks on this diet and nine were realimented three days more with diet I before being used in the determinations.

Diet I was adequate in all respects, whereas diet II only lacked a sufficient quantity of lysine to maintain body growth at any but the slowest rate, and diet III, although qualitatively adequate, was fed at a low level to prevent body growth. Animals on the pre-experimental diet received 200 mgm. of dried yeast daily, those on the experimental diets, 400 mgm., whereas 600 mgm. were fed animals being realimented or weighing more than 200 grams.

Tail blood was used for the weekly erythrocyte counts, and hemoglobin and total solids determinations, care being taken to obtain freely flowing blood but to prevent excessive loss. The hemoglobin determinations were made by the acid hematin method using a photolometer calibrated by the oxygen capacity method. Total solids were determined gravimetrically after drying in a vacuum oven (Gradwohl and Blaivas, 1920). Blood for the determination of the protein and non-protein nitrogen fractions was obtained from the abdominal aorta, with the animal under urethane anesthesia. The serum obtained after complete formation of the clot and centrifugation was used for the determination of total nitrogen, non-protein nitrogen, and serum albumin and globulin nitrogens. Non-protein nitrogen was determined upon serum after precipitation of the proteins by trichloracetic acid. The digest obtained by heating with sulfuric acid and selenium oxychloride was nesslerized and read in a photolometer. For the determination of total nitrogen, untreated serum digested with sulfuric acid, potassium sulfate and selenium oxychloride, was distilled in a micro-Kjeldahl apparatus, the ammonia caught in boric acid, and titrated with hydrochloric acid. Globulin was separated from the serum according to the method of Howe (1921) as modified by Robinson, Price and Hogden (1937; 1938) care being taken to prevent adsorption and loss of serum albumin; serum so treated permitted the determination of non-protein plus albumin nitrogens by the procedure described above for total serum nitrogen.

Results. The experimental data are presented in graphic form in figures 1 and 2 and are averages of values obtained on all the animals in each group, inasmuch as animals studied in the winter differed in nowise from those in the summer.

The growth of the rats in the various groups compared favorably with that obtained by Smith and Gross (1937) and expected changes in erythrocyte and hemoglobin levels were obtained (see fig. 1).

The growth of the rats in group I was accompanied by a rise in the average hemoglobin concentration from 12.5 grams per 100 cc. of blood at

38 days of age to 14.2 at 58 days of age. There was a concomitant rise in the average total blood solid concentration from 17.82 to 21.05 grams per 100 cc. The animals on the restricted diets showed greater increases of the hemoglobin levels and somewhat lesser gains in the total solid levels of the blood. Thus the lysine-deficient group showed an average hemoglobin concentration of 15.03 grams per 100 cc. of blood at 58 days of age and the calorie control group 15.13 grams at 59 days of age. These groups presented average total blood solid concentrations of 19.84 and 20.73 grams per 100 cc. respectively, in contrast to the 21.05 grams per 100 cc. of the normal group at the same age. On realimentation, as in the experi-

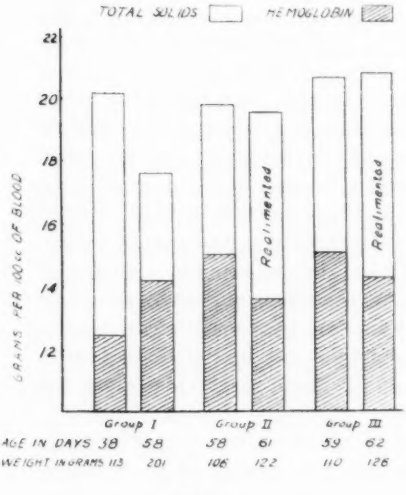

Fig. 1

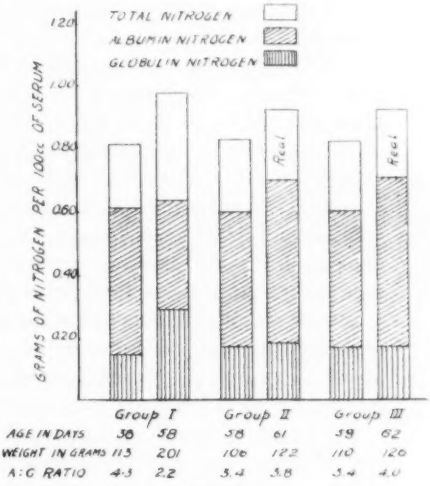

Fig. 2

Fig. 1. Mean values for total solids and hemoglobin concentrations
Fig. 2. Mean values for total nitrogen and protein partition of the serum

ence of Smith and Gross (1937) there was a fall in the hemoglobin levels, averaging 1.37 grams per 100 cc. in the case of the lysine deficient group and 0.77 gram in calorie control group. The total blood solid concentration was not changed significantly in either group (19.84 grams per 100 cc. of blood before, and 19.55 grams after realimentation for the lysine-deficient animals and 20.73 as against 20.85 grams per 100 cc. of blood for the calorie control animals). That the total blood solid concentration did not change upon realimentation is significant since it proves that simple hydration of the blood does not account for the fall in the hemoglobin and erythrocyte levels at this time.

The nitrogen fractions of the serum showed consistent relationships.

The non-protein nitrogen fractions throughout the experiment remain constant at 44 mgm. per 100 cc. of serum in all groups. The total nitrogen concentration in the normal group rose from 0.808 gram per 100 cc. at 38 days of age to 0.982 gram at 58 days of age, which compares favorably with results obtained by Swanson and Smith (1932) and Hatai (1918). This rise was due chiefly to an increase in the globulin fraction of the serum proteins (see fig. 2). The groups whose growth was restricted showed a barely perceptible rise in the total nitrogen of the serum, this being also due to a rise in the globulin fraction of the serum proteins. Upon realimentation, however, significant changes occurred. Both the lysine-deficient and calorie-control realimented groups showed a considerable rise in the total nitrogen concentration of the serum and in contrast to the situation existing in the normally growing animals, this rise was entirely due to an increase in the albumin nitrogen. During the three days of realimentation the level of total serum nitrogen increased from 0.829 gram per 100 cc. to 0.928 gram in the case of the lysine-deficient animals and from 0.827 to 0.921 gram per 100 cc. in the case of the calorie control animals. The albumin nitrogen concentration rose 0.091 gram from a level of 0.605 gram per 100 cc. of serum in the lysine-deficient realimented animals and 0.093 gram from a level of 0.609 gram in the case of the realimented calorie control group. These differences are statistically significant. It appears that the realimented animals although tending to approach the higher concentration of total serum nitrogen characteristic of normal animals of the same age, tend to revert to the characteristic blood picture of the younger animals of the same weight insofar as the protein nitrogen partition is concerned.

Discussion. Smith and Gross (1937) concluded, from the decrease in erythrocyte count and the parallel drop in the concentration of hemoglobin, accompanying the accelerated growth during realimentation, that, under the stress of filling the rapidly expanding vascular bed, the ability of the organism to produce the fluid part of the blood exceeds the ability to manufacture red blood cells. Their observations on the reticulocyte response, however, indicated a prompt and vigorous hematopoiesis. Insofar as they postulated a dilution of the erythrocytes with a fluid not containing these formed elements, they apparently were correct. However, with the further information now available from the present study, it appears that the diluting fluid is plasma, which contains somewhat more protein than does the plasma of the restricted animals before realimentation.

Of particular significance from the point of view of hemodynamics is the shift in the relationship of albumin to globulin in the plasma of the experimental animals within three days of realimentation. The data on the increase in total nitrogen and that in albumin nitrogen show a high degree of statistical significance whereas the change in globulin nitrogen is

not outside the realm of chance. Melnick, Field and Parnall (1940) have indicated that the A/G ratio is highly reproducible and that the relations demonstrated by the salting-out technique "are real and indicative of the relative amounts of these two independent protein systems in native serum," although, in the light of the transformation of large to small "molecules" of serum proteins in the ultracentrifuge after dilution (McFarlane, 1935), they question the validity of predicting oncotic pressure in vivo from the data on the A/G ratio. However, in view of the demonstrated relationship between the albumin fraction of the serum proteins and the development of clinical and experimental edema on the one hand and the maintenance of blood volume on the other (see Weech, 1938–1939) the changes in the present study indicate the direction of the functional adaptation to the circulatory emergency created by the extremely rapid growth; not only is more fluid mobilized in the plasma but its composition is altered in the direction of relatively increased concentration of the serum protein fraction which is osmotically more active, namely, the albumin, and thus fluid is more efficiently held in the blood vessels. It is also obvious that, under the experimental conditions employed, little, if any, difference in the influence on the response to realimentation was demonstrated between retardation of growth through lack of dietary lysine or through low energy intake.

SUMMARY

Restriction of growth, as measured by body weight and produced either by deficiency of food energy or of the essential amino acid lysine, retards the usual progress of changes in the concentration of the serum proteins in the young albino rat. The usual rise in serum globulin is observed both in the normally growing animal and in the animal stunted by either of the nutritional deficiencies employed although to a much less extent in the latter. Realimentation of the stunted animals results in an increase of total nitrogen of the serum which is largely due to serum albumin fraction.

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HORMONAL INFLUENCES ON THE WEIGHT OF THE ADRENAL IN INANITION

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The two distinct responses of the adrenal glands to inanition are dependent upon whether the starvation is complete or the inanition is chronic in type (Jackson, 1919; Mulinos and Pomerantz, 1940). The purpose of the present report is *a*, to determine the size, weight and appearance of the adrenal glands during the two types of inanition; *b*, to study the effects of certain hormonal preparations and of implants of pituitary glands upon the adrenal glands during inanition; and *c*, to discuss the evidence that during chronic inanition the hypophysis plays an important part in the genesis of the loss of weight of the adrenal glands.

EXPERIMENTAL. One hundred and eleven female and 81 male rats, each about 200 grams in weight, were employed in this investigation (table 1). Twenty additional rats were used in the pituitary implant experiments detailed in table 2. Ninety hooded rats (both sexes) were used as pituitary donors.

The general procedure used was that described previously (Mulinos and Pomerantz, 1940). *Complete starvation* was instituted by the withdrawal of food and water for from 7 to 13 days. *Chronic inanition* was induced by reducing to half (about 6 grams daily) the usual amount of a complete food which had been consumed during a preliminary control period. The animals were weighed daily. When the hormonal preparations were used, they were injected subcutaneously each day. Estradiole benzoate (Progynon-B) was given to the female rats in doses of 2 to 10 R.U. and invariably resulted in estrus. Pregnancy urine extract (Follutein) was administered in doses of 4 to 10 R.U. to the female and 4 to 25 R.U. to the male rats; an alkaline anterior pituitary extract (growth factor) was given to female rats in doses of 5 to 10 growth units and testosterone proprionate to a male rat in doses of 0.5 mgm.[1] Seven female and 15 male rats were castrated and

[1] Progynon-B and testosterone proprionate were kindly supplied by Dr. Erwin Schwenk of the Schering Corp. The Follutein and the alkaline extract of the anterior pituitary glands were generously furnished by Dr. H. S. Newcomer of E. R. Squibb and Sons.

divided into groups for further study of the effects upon the adrenal glands of inanition as compared with the fully fed controls. Thirteen of the male rats received in addition testosterone proprionate in daily doses of 0.1 to 0.2 mgm. subcutaneously for six days. The pituitary implants were taken from normal rats which were killed by decapitation.

At the termination of each experiment the rats were lightly anesthetized with ether and exsanguinated from the heart. The organs were dissected and weighed on an analytical balance. Rats that died during an experiment were discarded.

RESULTS. The data are presented in table 1. In *complete inanition* the weight of the adrenal glands is dependent upon the length of time of the starvation and upon the percentile loss in body weight. At the end of the first week of starvation the adrenal glands are usually heavier, although they may be normal and at times reduced in weight. As the period of starvation lengthens and the body weight loss becomes more severe the adrenal glands increase in weight. When the starvation was allowed to continue until the rats became moribund the adrenal glands were invariably enlarged. In contrast to the firm yellow glands of the normally fed controls, those of the starved rats were large and red with a cortex that was friable and congested with blood. The adjacent peritoneal tissues were often edematous.

Chronic inanition induced in 9 male and 20 female adult rats, resulted in a progressive atrophy of the adrenal glands which was approximately proportional both to the duration of the inanition and to the percentile loss in body weight. The adrenal glands of male rats are normally smaller than those of female rats of corresponding body weight or age. In our series, the adrenal glands of the male rats averaged 28.1 mgm. in weight as compared with 47.4 mgm. for those of the female rats. This difference is only in part accounted for by the fact that female rats weighing 200 grams are about 2 months older than males of equal weight. These figures agree with the data of Donaldson (1924). The loss of weight of the adrenal glands of the female rat was more marked (36 per cent) than the body weight loss (23 per cent); while in the male it was less (19 per cent) than the body weight loss (33 per cent). During chronic inanition the female-male differential of adrenal weight was 7.2 mgm. as compared with a differential of 19.3 mgm. of the normally fed rats. The adrenal glands of the female rat lost more in weight than those of the male rat, despite the fact that the males lost a greater percentage of their body weight. The adrenal glands of chronically starved rats gain in weight when the rats are fully fed again, and attain normal weight when the loss in body weight has been recovered (table 1). When the chronically underfed rat is in addition subjected to complete starvation, its atrophic adrenal glands gain in weight as already described under complete inani-

tion. The small, firm and yellow glands become larger, softer and darker in color

As for the normal adrenal gland (Selye, 1937), so during chronic inanition, noxious stimuli are capable of producing enlargement of the adrenal

TABLE 1

The weight of the rat adrenal glands as related to the type of inanition and as affected by hormonal injection or castration

STATE OF NUTRITION	DURATION	NUMBER AND SEX	SPECIAL PROCEDURE INSTITUTED	BODY WEIGHT CHANGE	ADRENAL GLAND WEIGHT
	days			*per cent*	*mgm.*
Fully fed controls		20 F	None		47.4 (39–52)
		4 F	Castrated 200 days	+33	33.6 (31–42)
Complete starvation	8.2	15 F	None	−37	54.3 (39–70)
	9.0	5 F	Inject. crude liver extract	−39	65.0 (45–79)
	8	10 F	Estrogen or P.U.	−37	56.6 (46–74)
	8	15 F	"Growth hormone"	−37	61.0 (50–75)
Chronic inanition	106	20 F	None	−23	30.0 (23–39)
	56	5 F	Refed	−18–+12	47.6 (46–49)
	81	5 F	Complete starvation terminally 4 days	−20–−40	35.6 (30–43)
	96	2 F	Spont. refusal of food until moribund	−38	54.0 (50–58)
	28–65	7 F	"Growth hormone" and/or P.U.	−27	36.0 (32–39)
	200	3 F	Castrated 225 days	−17	25.5 (22–28)
Fully fed controls		10 M	None		28.1 (25–31)
		5 M	Castrated*	+24	35.5 (32–39)
Complete starvation	7	7 M	None	−32†	34.1 (25–43)
Chronic inanition	72	9 M	None	−33	23.8 (21–27)
	65	3 M	Refed	−37–+14	30.6 (25–33)
	41–104	37 M	P.U. or testosterone	−29 (12–44)	24.8 (18–33)
	55	10 M	Castrated* 68 days	−34	23.6 (19.6–27)

* These groups include the rats which received injections of testosterone proprionate daily for 6 days, without effect upon the adrenal glands.

† Rats weighed 277 grams before starvation.

glands. Such stimuli may be in the form of injections of foreign materials, some disease process, a drug, etc. Thus in almost all cases where non-adrenotropic hormonal preparations or tissue extracts were injected, or when the animal became ill and refused food, the glands were larger than the controls (table 1). Obviously, the effects of illness and of such noxious

substances cannot be interpreted as being directly adrenotropic. Sufficiently noxious stimuli may cause an increase in the production of adrenotropic hormone by the hypophysis (Selye, 1937) or the adrenals may be rendered more sensitive to the adrenotropic hormone already present in in the animal even though this may be reduced in quantity.

An analysis of the conditions associated with the weight changes of the adrenal glands during inanition involved the following possibilities: *a*, the effect of inanition upon the glands directly; *b*, an alteration of pituitary adrenotropic hormone concentration in the blood due to hypophyseal inanition; and *c*, a diminution of gonadotropic and sex-hormone concentrations. The following experiments suggest that the adrenal weight changes from inanition are not due to a decrease in sex hormone concentration, and that the atrophy of the glands during *chronic* inanition is primarily due to a fall of the adrenotropic activity of the hypophysis.

The effect of gonadotropic hormone (P.U.); anterior pituitary extract; estradiole benzoate; testosterone proprionate; or of castration upon the weight of the adrenal glands during inanition. It has been shown that there is a deficiency of gonadotropic hormone during inanition. This deficiency is believed to be due to a failure of the gonadotropic function of the hypophysis (Mulinos et al., 1939; Werner, 1939). In consequence, the gonads would secrete fewer sex hormones. Because of the alleged functional relation between the gonads and the adrenal glands (Hall and Korenchevsky, 1938) it was necessary to determine the rôle played by these hormonal deficiencies in the origin of the adrenal gland weight changes during inanition.

Twenty-five female rats were starved completely and during this period were injected with estradiole benzoate, P.U., "growth hormone," or combinations of the latter two. These substances when injected in the doses already mentioned did not influence the gain in the weight of the adrenal glands which occurred from the complete starvation (table 1).

The hormones were also injected into 43 chronically underfed rats before and during the inanition, or after the inanition had progressed to a marked degree. The chronic inanition resulted in an atrophy of the adrenal glands despite the injection of these hormones. The potency of the hormones was gauged by the appearance of estrus and the increase of ovarian and adnexal weights in the female rats and by the increase in size and weight of the accessory sex organs in the males.

Castration of the female rat resulted in a reduction of the weight of the adrenal glands to 33.6 mgm. as compared with 47.4 mgm. for normal females (see also Hall, 1940). When chronic inanition was induced for 200 days in the castrated female rat the weight of the adrenal glands fell to 25.5 mgm. as compared with 30.1 mgm. for the chronically underfed uncastrated controls.

Castration of the male rat, in contrast to the female, resulted in adrenal

glands which weighed 35.3 mgm. as compared to 28.1 mgm. for normal rats (see also Hall and Korenchevsky, 1938). Chronic inanition for 55 days reduced the weight of the adrenal glands in both castrated and noncastrated male rats to 23.7 and 23.6 mgm. respectively. The injection of small doses of testosterone into castrated male rats had no effect upon

TABLE 2

The effect of pituitary gland implants upon the weight of the adrenal glands during chronic inanition

CONTROL GROUPS					PITUITARY IMPLANT GROUPS							
							Adrenals		Ovaries		Thyroids	
Rat number	Body weight loss	Adrenals, weight	Ovaries, weight	Thyroids, weight	Rat number	Body weight loss	Weight	Per cent deviation from control average	Weight	Per cent deviation from control average	Weight	Per cent deviation from control average
Experiment I, February to July												
Underfed 151 days. No implants					Underfed 152 days. 8 pituitary implants from ♂ and ♀ donors							
	per cent	*mgm.*	*mgm.*	*mgm.*		*per cent*	*mgm.*		*mgm.*		*mgm.*	
43-1	21	30	25	14.0	44-1	25	38	+26	30	+20	13.5	−3
43-4	25	37	37	14.5	44-2	28	40	+33	39	+56	10.0	−28
43-5	30	26	13	13.0	44-3	38	40	+33	38	+52	19.0	+36
45-1	25	24	27	17.0	44-4	28	43	+43	38	+52	11.0	−21
45-3	32	33	22	11.0	44-5	37	32	+7	21	−16	13.0	−4
Av.	27	30.0	25.0	13.9		31	38.6	+29	33.2	+33	13.3	−4
Experiment II, August to November												
Underfed 114 days. 10 brain implants					Underfed 113 days. 10 pituitary implants from ♀ donors							
71-1	29	38.0	23.3	13.8	72-1	34	47.4	+40	37.8	+136	13.8	−4
71-2	32	29.8	15.6	16.7	72-2	37	49.7	+46	35.0	+119	10.3	−28
71-3	37	36.3	11.7	11.5	72-3	31	38.8	+14	33.2	+107	11.3	−21
71-4	36	32.5	19.1	16.8	72-4	35	50.0	+47	37.3	+133	12.0	−16
71-5	31	33.3	10.5	13.3	72-5	39	55.6	+64	20.0	+25	9.4	−34
Av.	33	33.9	16.0	14.4		35	48.3	+42	32.6	+104	11.4	−21

the weights of the adrenal glands of either the chronically underfed or the fully fed groups. The testosterone was given for only 6 days but proved sufficiently potent to cause growth of the accessory sex organs.

Since the responses of the adrenal glands to inanition occurred in spite of the fact that these hormones were supplied artificially, it may be con-

cluded that the gain in weight of the adrenal glands during complete inanition, and the atrophy during chronic inanition, were not due to any deficiency of the sex or growth hormones which may have occurred during the inanition.

The effect of pituitary gland implants upon the weight of the adrenal glands during chronic inanition. The following experiments demonstrate that chronic inanition depresses the adrenotropic function of the pituitary glands as well as the gonadotropic. The results are summarized in table 2.

Two groups of 5 female rats were underfed for about 5 months. Each of the rats of one group received 8 whole pituitary glands from normal female and male donors. The injections were made into the hind leg muscles over a period of 10 days. Three days after the last injection both this group and the control group were autopsied. The efficiency of the pituitary implants was judged by the effects upon the weights of the sex organs (table 2, expt. I). The experiment was repeated upon a second group, the controls of which received intramuscularly a piece of brain tissue from the donor rats. This was done in order to obviate the chance that infection or trauma contributed to increase the weight of the adrenal glands in the pituitary implant group. Each of these rats received 10 implants, 5 rats receiving hypophyses of female donors, and 5 a piece of brain tissue (table 2, expt. II). At autopsy there was no evidence of infection or local tissue reaction. Among the 10 rats of the pituitary implant group, the adrenal glands of but one rat (44-5) fell within the range of control weights; all others had increased in weight above the controls.

It is probable that the weight increase of the adrenal glands of the pituitary implant groups was due to the adrenotropic content of the implanted hypophysis. It is believed that the thyroid glands played no important part in this phenomenon because in the rats which received the hypophyseal implants the thyroid glands were no heavier than, and histologically did not differ from, the controls (compare Emory and Winter, 1934) (table 2).

The results of the pituitary implant experiments suggest that the atrophy of the adrenal glands which accompanies chronic inanition is due primarily to a relative diminution of the adrenotropic hormone in the blood rather than to the direct effects of the malnutrition upon the adrenal glands.

SUMMARY

Complete starvation in adult rats resulted in an increase in the weight of the adrenal glands. In contrast, chronic underfeeding resulted in a loss of weight of the adrenal glands usually relatively greater than the loss in body weight. Underfed rats which were refed and allowed to attain their normal body weight, at autopsy revealed adrenals of normal weight.

During complete or chronic inanition the possible fall in the concentration of gonadotropic hormone (P.U.), "growth hormone," estrogen, or testosterone, is not a major factor in affecting the weight of the adrenals. When injected in small physiologically active doses, these hormones do not markedly alter the gain or loss of the adrenal weight of the animals.

Despite continued underfeeding, pituitary glands of normal rats implanted into chronically underfed female rats resulted in a gain in the weight of the atrophied adrenal glands. This indicates that the atrophy of the adrenal glands was not due directly to the malnutrition, but at least in part to an insufficient amount of adrenotropic hormone. It is suggested that in the chronically underfed rat, this insufficiency of adrenotropic hormone has its origin in the physiologically depressed function of the pituitary gland.

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THE EFFECT OF BILE ACIDS ON HEPATIC BLOOD FLOW

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The relationship of hepatic blood flow to bile formation has not been extensively studied (1). Schwiegk (2) has observed that the choleresis produced by the intravenous injection of sodium dehydrocholate (Decholin-sodium) is associated with a 100 per cent increase in hepatic arterial blood flow and a variable effect on portal venous flow; apparently only one animal was injected once.

This work was undertaken to ascertain if Schwiegk's observation could be confirmed and, if so, whether other bile acids had a similar effect. The results would have a bearing on the broader question of the mechanism of choleresis.

EXPERIMENTAL METHODS. In order to measure the rate of blood flow, it was decided to use the direct current thermostromuhr introduced by Baldes and Herrick (3). Before using this method, however, it was necessary to conduct extensive preliminary experiments to determine whether or not this instrument was reliable in our hands.

We have made repeated calibrations on the same thermostromuhr unit, as well as on other units, both *in-vitro* and *in-vivo*. The calibrations *in-vitro* were made by the gravity perfusion method using different vessels, different temperatures, and different heating intensities. Fresh citrated dog's blood was used as the perfusing fluid. In the calibrations made *in-vivo* the unit was placed proximally on the femoral artery. Heparin or chlorazol fast pink was injected as an anticoagulant. The artery was then cannulated 10 to 12 cm. distal to the unit, all intervening branches being ligated. The dog was bled through the arterial cannula, the flows being timed and measured. The blood pressure was kept fairly constant by returning blood into the opposite femoral vein.

The results of calibration. The essential results obtained during the calibration of one unit are shown in figure 1. Curve 1 was obtained *in-vitro* at 25°C. with a heater current of 620 ma. and was fitted to 70 experimental points by the method of least squares. Seven different arteries (5 carotids and 2 hepatics) were used in obtaining the data and 2 months elapsed between the first and last calibrations. The standard

error of estimate for a single observation in the prediction of V from G is ± 15 cc. per minute. The average per cent error over the entire range is 7 per cent. In curve 2, the points designated *X* were obtained with a

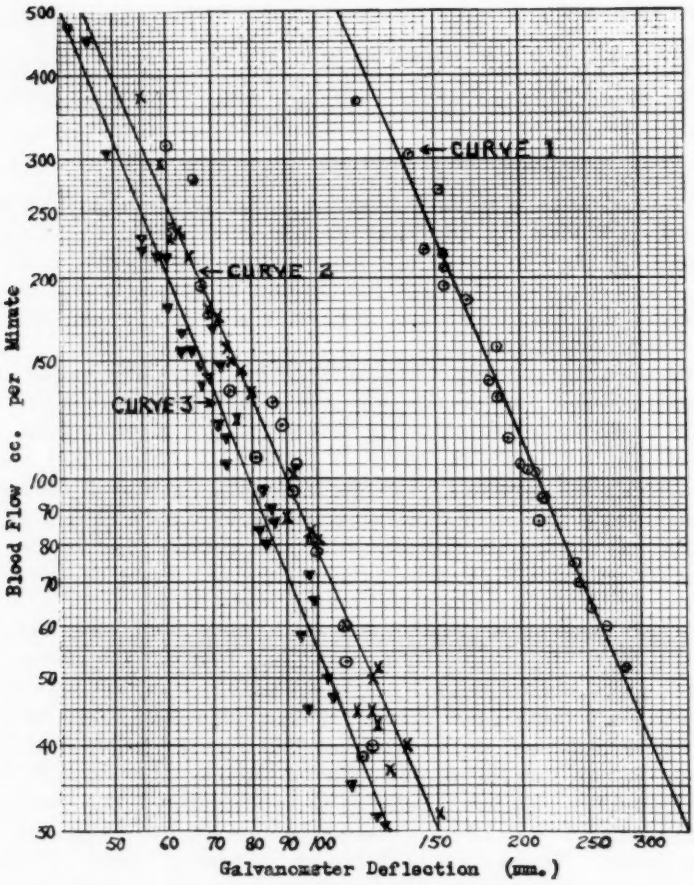

Fig. 1. Calibration curves for thermostromuhr unit E-4. Curve 1, *in-vitro*, 620 ma., 25°C. Curve 2, *in-vitro*, 400 ma., 25°C. indicated by *X*, 38°C., indicated by *O*. Curve 3, *in-vivo*, 400 ma., the data being obtained from 5 dogs.

heater current of 400 ma. *in-vitro* at 25°C. The curve was fitted to 60 experimental points by the method of least squares. Four different arteries (3 carotids, 1 hepatic) were used and 2.5 months elapsed between the first and last experiments. It will be noted that curves 1 and 2 have

the same slope and that the galvanometer deflections for any given flow are exactly proportional to the square of the respective heater currents. These facts give additional evidence for the reliability of the results. The standard error of estimate on this curve is ± 15 cc. per minute and the average per cent error is 7 per cent. The points designated by a dot within a circle on curve 2 represent data obtained *in-vitro* with a heater current of 400 ma. and a temperature of 38°C. The curve fitted to 51 such points is exactly superimposed on that obtained at 25°C. Curve 3 was obtained *in-vivo* with a heater current of 400 ma. and was fitted to 80 experimental points obtained from 5 different dogs. The standard error of estimate on this curve is ± 20 cc. per minute and the average per cent error is 10 per cent.

The preparation of the animals. In the bile acid studies acute experiments were performed on dogs under sodium pentobarbital anesthesia. Carotid blood pressure was recorded. The common bile duct was cannulated and the cystic duct ligated. The blood vessel to be studied was carefully dissected clean for a sufficient distance to allow application of the stromuhr unit. The unit was applied to the hepatic artery near the origin of the vessel as it arches in the border of the lesser omentum. It thus measured not only the blood going to the liver but also that going through the gastroduodenal branch. This latter vessel was not ligated because we wished to maintain normal circulation insofar as possible and to avoid any possibility of setting up eddy currents. The unit had to be adjusted carefully to avoid kinking of the vessel. On the superior mesenteric artery, the stromuhr was applied close to the origin of the vessel where it is free of branches. The correctness of the fit was tested by observing whether a steady zero point was maintained with no heat applied and also by noting whether *the galvanometer deflection with heat applied was proportional to the square of the heater current.* The dog remained "idle" for 30 minutes after completing all operative procedures. Then, after suitable control periods, recording blood pressure, bile flow, and blood flow, the effects of various bile acids as salts were determined.

The following salts were tested: sodium dehydrocholate[1] (Decholin-sodium) in 0.5 gram doses in 20 per cent solution; mixed triketocholanates[2] (Ketochol) in 0.25 gram doses on 10 per cent solution; pure sodium cholate[1] in 0.5 gram doses in 10 per cent solution; sodium taurocholate[3] in 0.5 gram doses in 10 per cent solution; and sodium glycocholate[4] in 0.5 gram doses in 10 per cent solution. All injections were given into the femoral vein and *at a rate that did not alter blood pressure, except as noted under results.*

[1] Riedel-de Haen.
[2] Searle and Company.
[3] Natural product (Riedel-de Haen) and a synthetic product (Searle).
[4] Synthetic (Searle).

TABLE 1

Effect of sodium dehydrocholate on hepatic arterial blood flow

DOG NUMBER	WEIGHT	WEIGHT LIVER	BLOOD FLOW		PER CENT CHANGE	CC./MIN./100 GM. LIVER (CONTROL)
			Control	Decholin		
	kgm.	*grams*	*cc./min.*	*cc./min.*		
1	22.7	680	215	275	+28	29
2	23.0	690	55	175	+218	11
			80	125	+56	
3	25.0	750	76	200	+163	20
			150	150	0	
			150	150	0	
4	25.0	750	185	265	+43	25
			190	210	+10	
5	20.0	600	150	200	+33	18
			105	175	+67	
			62	94	+51	
6	18.2	550	125	250	+100	18
			60	76	+27	
			43	58	+35	
7	9.1	275	74	90	+22	25
			66	80	+21	
			64	80	+25	
8	16.0	480	160	200	+25	34
			180	265	+47	
9	15.9	480	97	150	+54	21
			97	145	+50	
10	13.2	395	51	67	+31	19
			72	83	+15	
			76	97	+28	
			98	129	+32	
11	16.4	490	132	203	+54	30
			175	230	+31	
			160	245	+53	
12	22.7	680	255	480	+88	44
13	18.1	540	80	92	+15	19
			68	83	+22	

TABLE 1—*Concluded*

DOG NUMBER	WEIGHT	WEIGHT LIVER	BLOOD FLOW		PER CENT CHANGE	CC./MIN./100 GM. LIVER (CONTROL)
			Control	Decholin		
	kgm.	*grams*	*cc./min.*	*cc./min.*		
14	16.4	490	100	100	0	20
			100	100	0	
15	34	1000	245	310	+26	25
			180	315	+70	
			180	430	+139	
16	16	480	80	96	+20	17
			78	94	+20	
17	17.3	520	240	275	+15	32
			150	180	+20	
18	16	480	185	260	+37	50
19	17.7	530	125	200	+60	24
20	16	480	100	150	+50	21
			110	170	+54	
21	23.2	690	195	245	+26	26
			84	110	+31	
Averages			126	186	+48	

Photographic records[5] as well as direct readings of the deflection of the galvanometer were made.

RESULTS. *Hepatic arterial blood flow.* A. *Magnitude of normal flow.* The average normal flow through the hepatic artery of 28 dogs was 26 cc. per minute per 100 grams of liver with a range of 11 to 50 cc. per minute (table 1).

B. *Effect of intravenous sodium dehydrocholate.* We have performed a total of 46 experiments on 21 dogs (table 1). In 42 instances (20 dogs) an increase in blood flow was observed ranging from 15 to 218 per cent with an average of +48 per cent. The maximum increase in flow was reached within 5 minutes after the injection was started and the rate gradually decreased to its original level in about 15 or 20 minutes. In some animals, the increase lasted much longer (40 min.) and in others it was more transient (10 min.). In a few instances, the injection was given too rapidly and some fall in blood pressure occurred. In these cases, the

[5] The photographic records and other detailed data were demonstrated at the New Orleans Meeting of the American Physiological Society, March, 1940.

blood flow response was more rapid and tended to be greater than when the blood pressure did not change. An average bile flow increase of 700 per cent was observed. The maximum rate of bile flow was reached about 2 minutes after the maximum blood flow and the rate remained increased

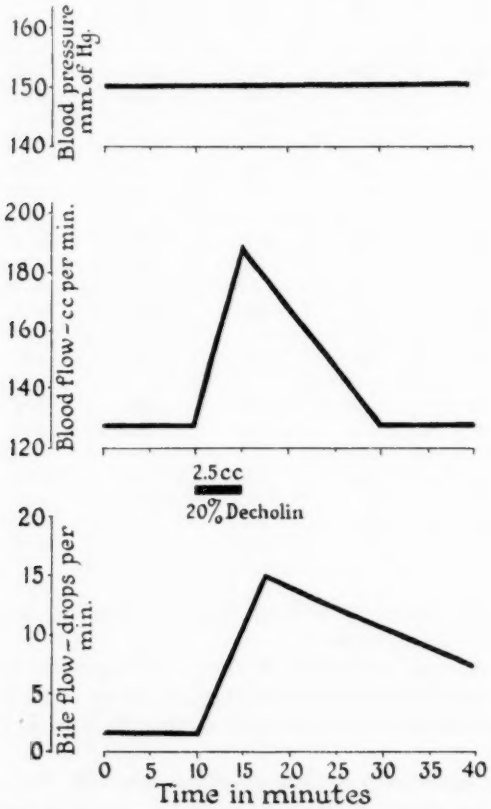

Fig. 2. Showing the effect of sodium dehydrocholate on hepatic arterial blood flow, bile secretion and blood pressure. The graphs represent the average values of 42 tests on 20 dogs.

for a longer period than did the blood flow. To conserve space the results on blood pressure, hepatic blood flow and bile flow were averaged and graphed, and are shown in figure 2.

In 4 experiments on 2 dogs, no change in blood flow was observed. In 2 of these cases, the secretory response was very slight. The other dog was totally refractory and showed neither an increase in bile flow nor in blood

flow. In 1 dog (not in table 1) whose postoperative condition was poor (blood pressure close to shock level), we injected the bile salt during a time when the blood pressure was gradually falling (to a low of 40 mm. of Hg) and observed a decrease of 50 per cent in hepatic arterial blood flow which paralleled the fall in systemic pressure. Nevertheless there was a marked increase in bile flow (800 per cent).

C. *Effect of mixed triketocholanates* (*ketochol*). Sixteen experiments were performed on 6 dogs (table 5). In 14 cases (6 dogs) an increase in blood flow ranging from 15 to 119 per cent with an average of 39 per cent was observed. The time relations and the bile flow responses were similar to those observed with sodium dehydrocholate.

In 2 experiments (2 dogs) no change in blood flow was observed, though an increase in bile flow occurred.

D. *Effect of sodium cholate.* Twenty-seven experiments on eleven dogs were performed (table 2). The results with this particular salt were quite variable. In 11 cases, no change in blood flow was observed. In 10 cases, a decrease in flow ranging from −16 to −55 per cent was observed (average—31 per cent). In 6 cases, an increase in flow from 19 to 86 per cent (average—47 per cent) was noted. There did not seem to be any correlation between the blood flow response and the degree of choleresis produced. In all but one dog sodium dehydrocholate was also injected and this salt produced an increase in blood flow in all except one animal. The latter was totally refractory to any bile salt both in regard to blood flow and bile flow. In any given dog in which sodium cholate produced an increased blood flow, sodium dehydrocholate produced a greater increase except in one case.

E. *Effect of conjugated cholates* (table 5). In 9 tests on 5 animals, *sodium taurocholate* produced a decrease in hepatic arterial flow in 6 cases. The average decrease was 16.5 per cent. In 3 instances no change occurred. *Sodium glycocholate* produced a decrease in hepatic arterial flow in 4 of 6 tests on 5 dogs. No change occurred in 2 cases.

Portal venous flow. A. *Average normal flow.* The average normal flow in 6 dogs was 18 cc. per kilo per minute, or 60 cc. per minute per 100 grams of liver.

B. *Effect of sodium dehydrocholate* (table 3). Fifteen experiments on 6 dogs were performed. In 8 cases (4 dogs) a decrease in portal blood flow was observed ranging from 10 to 44 per cent with an average of 29 per cent. In 5 experiments (3 dogs) an initial decrease in flow followed by an increase was observed. The initial decrease ranged from 14 to 43 per cent, averaging 23.4 per cent. The subsequent increase ranged from 21 to 46 per cent with a mean of 32.5 per cent. In 1 experiment, an increase in flow of 38 per cent was observed without any previous decrease. In 1 dog there was too much spontaneous variation to draw any conclusions.

Superior mesenteric artery. A. *Effect of intravenous sodium dehydrocholate.* Fourteen experiments were performed on 7 dogs. In 10 cases

TABLE 2

Effect of sodium cholate on hepatic arterial flow

DOG NUMBER	WEIGHT	Na CHOLATE BLOOD FLOW		PER CENT CHANGE	DECHOLIN BLOOD FLOW		PER CENT CHANGE
		Control	Na Cholate		Control	Decholin	
	kgm.	*cc./min.*	*cc./min.*		*cc./min.*	*cc./min.*	
1	22.7	310	138	−55	255	480	+88
		150	94	−37			
2	18.2	110	110	0	80	92	+15
		98	98	0	68	83	+22
		68	97	+42			
3	16.3	96	96	0	100	100	0
		96	96	0	100	100	0
4	34	430	300	−30	245	310	+27
		310	310	0	180	315	+65
					180	275	+55
5	17.7	240	120	−50			
		215	400	+86			
		220	380	+73			
6	15.9	165	100	−39	80	96	+20
		150	110	−27	78	94	+20
		74	62	−16			
7	17.3	275	275	0	240	275	+15
		215	180	−16	150	180	+20
		150	150	0			
8	15.9	215	215	0			
		260	260	0	190	280	+37
9	17.7	105	125	+19	125	200	+60
		180	180	0			
10	14.5	127	170	+33	100	150	+50
		105	135	+28	110	170	+54
11	23.2	180	180	0	195	245	+26
		150	110	−27	84	110	+31
		105	86	−18			

(5 dogs) a decrease in blood flow was observed ranging from 8 to 49 per cent with an average of 22 per cent. In 2 cases (1 dog) an initial

decrease averaging 34 per cent was followed by a 22 per cent increase. In 2 cases no change occurred (table 4).

Effect of a slow continuous injection of sodium dehydrocholate into a mesenteric vein. Having determined that the relatively rapid injection of a concentrated sodium dehydrocholate solution into a peripheral vein produced a marked increase in hepatic arterial blood flow, the question arose, does the slow absorption of bile salts from the intestine such as occurs

TABLE 3

Effect of sodium dehydrocholate on portal venous flow

DOG NUMBER	WEIGHT	BLOOD FLOW		PER CENT CHANGE
		Control	Decholin	
	kgm.	*cc./min.*	*cc./min.*	
1	7.7	180	100	−44.5
		130	74	−43
			190	+46
		80	60	−25
			112	+40
2	9.1	82	74	−10
		100	60	−40
		63	52	−18
			80	+27
3	9.1	160	98	−39
		150	107	−29
		130	98	−25
4	10	70	60	−14
			90	+28
		52	43	−17
			63	+21
		55	76	+38
5	10.9	400	310	−22.5
		400	260	−35

normally following the flow of bile into the intestine in response to a meal also produce such a blood flow response?

With this in mind we have observed the effect of a slow, continuous injection of a dilute (1 per cent) solution of sodium dehydrocholate into a mesenteric vein. The animals were prepared as previously described. In addition, a small mesenteric vein was cannulated and connected with a drip apparatus. After a suitable control period, the venoclysis was started. The rate was so regulated that the animal received 0.5 gram of bile salt per hour (50 cc./hour). This is about the rate at which Schmidt, Beazell, Berman, Atkinson and Ivy (4) returned bile to the duodenum in their

studies on chronic biliary fistula dogs. The drip was continued for 1.5 hours and the dogs were observed for 0.5 to 1 hour after the infusion was stopped.

Four dogs were studied in this way (fig. 3). Two of these showed an increase in hepatic arterial blood flow which tended to return to the control level shortly before or shortly after the drip was stopped. The other two

TABLE 4

Effect of Na dehydrocholate on the blood flow in the superior mesenteric artery

DOG NUMBER	WEIGHT	BLOOD FLOW		PER CENT CHANGE
		Control	Decholin	
	kgm.	*cc./min.*	*cc./min.*	
1	20.4	245	225	−8
		250	180	−28
		175	157	−10
2	29.5	135	135	0
		150	160	+7
			125	−17
3	23	400	310	−22
4	15	300	235	−29
			410	+24
		285	145	−49
		215	130	−39
			255	+19
5	20	180	110	−39
		78	62	−20.5
6	18.2	340	290	−14.7
		185	130	−27
7	10	86	86	0

showed an increase in flow which was maintained for 40 to 50 minutes after the drip had been stopped.

In view of the fact that Walker, Schmidt, Elsom and Johnston (5) in their studies on renal blood flow employing the thermostromuhr technique have reported a tendency for the galvanometer to "drift" spontaneously toward the direction of an increased flow during the course of an experiment, we felt it important to have a control series of animals which were observed over a similar period of time. Four dogs were used as controls (fig. 3). Continuous observation of hepatic arterial blood flow was made over a period of from 1 to 2.5 hours. In 3 animals a slow mesenteric drip

of 0.9 per cent NaCl was used instead of the bile salt solution; in one animal nothing was done beyond observing the blood flow.

If the average curve of the control series is compared with the average curve of the treated series, a definite difference is noted. In none of the control animals was a persistent spontaneous "drift" of the galvanometer toward a more rapid flow noted. The few spontaneous increases which were observed were of relatively short duration and never lasted as long as those seen in the treated animals (1.5 to 2 hrs.). In one animal we noted a sudden swing of the galvanometer almost back to zero followed by as sudden a return to its former reading, all within 1.5 to 2 minutes. This type of deflection can be produced by a sudden temporary kinking of the vessel.

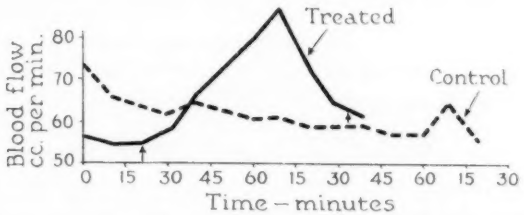

Fig. 3. Showing the effect of slow continuous injection of sodium dehydrocholate into a mesenteric vein on hepatic arterial blood flow; *T*, treated and *C*, control. The graphs represent the average values of 4 tests on 4 dogs.

Discussion. Few data and instructions are available regarding the accuracy and the calibration of the thermostromuhr. The accuracy indicated by our results compares well with that (8 to 10 per cent) reported by others (6–9).

We were particularly interested in comparing our curve obtained *in-vitro* with that obtained *in-vivo*. When these curves are compared by statistical methods, which are not well adapted for comparing this type of data, it is found that there is no significant difference between them. In spite of this fact, however, the flows obtained *in-vivo* are consistently lower than those obtained *in-vitro* and one wonders whether this is a real difference due to conditional variations or whether it is due to the technical difficulties of obtaining calibrations *in-vivo*. From our experience we believe it is due to the latter. It is difficult to maintain a steady rate of flow in *in-vivo* calibrations whereas this is readily accomplished *in-vitro*. In the former case, the rate of flow tends to decrease with time and any lag in the galvanometer would give a reading too low for the observed flow. Another source of error is the possibility of turbulence produced by the ligation of the arterial branches.

Walker, Schmidt, Elsom and Johnston (5) believe that *in-vitro* calibra-

tions are invalid when applied to measurements on the experimental animal and indeed that even *in-vivo* calibrations cannot be applied from animal to animal. This possibility does not materially concern our conclusions, since we record a control flow, a test flow and a post-test flow, and compare the differences. We do not claim that the figures for the volume flows recorded here are absolute—the thermostromuhr is not a precision instrument. That they are relatively accurate, however, is indicated by the fact that our values for "normal" hepatic flows agree well with those obtained by direct methods (10–12).

For the purposes of comparison we had to be concerned principally with such items as uniformity of contact of the stromuhr with the vessel, turbulence and kinking. If the unit used is carefully selected as to size, and if certain tests are applied several times during the course of an experi-

TABLE 5

	HEPATIC ARTERY					PORTAL VEIN Na DEHYDROCHOL.	SUP. M. ARTERY Na DEHYDROCHOL.
	Na dehydrochol. (Decholin)	Mixed dehydrochol. (Ketocholl)	Na cholate	Na tauroch.	Na glycochol.		
Number of dogs	21	6	11	5	5	6	7
Number of tests	46	16	27	9	6	15	14
Increased flow	42	14	6	0	0	1	0
No change	4	2	11	3	2	1	2
Decreased flow	0	0	10	6	4	8	10
Initial decrease to increase	0	0	0	0	0	5	2

ment we do not believe that the factor of contact should yield erroneous results. If the zero point of the galvanometer with no current passing through the heating unit fluctuates to any degree, one must assume that at times one of the thermojunctions has lost proper contact with the vessel wall. Another test is whether or not the galvanometer deflection is proportional to the square of the heater current at any given flow. The results of experiments in which these tests were unsatisfactory were disregarded in our work. In our experiments we were not concerned with very slow or rapid rates of flow. Our *in-vitro* and *in-vivo* tests, however, revealed that with the units we used very little reliance could be placed on flows of less than 20 cc. or greater than 400 cc. per minute (13, 14), and that the thickness of the wall of the vessels we used did not modify the results beyond the limits of error of the method.

The oxidized unconjugated bile salts produced a decided increase in hepatic arterial flow when injected intravenously. The fact that a marked choleresis was produced in 1 dog in which the hepatic arterial flow de-

creased along with a drop in systemic blood pressure indicates that sodium dehydrocholate can produce a choleresis independent of an increase in arterial flow. Usually, however, the choleresis produced by the oxidized cholates is associated with an increase in arterial blood flow. In this connection we have tested the effects of *cinchophen* on hepatic arterial flow because this substance is as effective an *hydrocholeretic* in the dog as sodium dehydrocholate (15). In doses of 50 mgm. per kilo intravenously this compound produced an increase of 47 per cent in hepatic arterial flow in each of two tests. This suggests that any true hydrocholeretic, a substance that increases the flow of bile and decreases both its content of solids and its viscosity, may increase hepatic arterial flow. Since an increase in arterial flow is observed when a slow injection of sodium dehydrocholate is given into a mesenteric vein to simulate the absorption of bile salts from the intestine during digestion, it may be that some of the therapeutic effects claimed for the oral administration of various oxidized bile salt preparations in hepatic insufficiencies are due in part to an increase in arterial flow to the liver.

It should be noted, however, that the total blood flow to the liver was not significantly increased by dehydrocholic acid and the unconjugated ketocholanates. As a rule, the increase in the total volume flow through the hepatic artery equaled the decrease in total volume flow through the portal vein. The hydrocholeresis was presumably associated with an increased oxygen supply to the liver. Whether this indicates that hydrocholeresis *per se* increases the oxygen demand of the hepatic cells or that such substances as ketocholanates and cinchophen are metabolized in such a manner as to increase oxygen demand is at present conjectural. The unconjugated and conjugated cholates, which are choleretic but not hydrocholeretic (16, 17), did not significantly affect hepatic arterial flow. Thus it is certain that unconjugated ketocholanates and cinchophen affect hepatic blood flow differently from cholates. And, in regard to glandular physiology, it is of special interest that the liver can secrete, in response to bile salts, an increased amount of secretion without the occurrence of a significant increase in total blood flow.

Although it was just indicated that ketocholanates and cinchophen may increase hepatic arterial flow by increasing oxygen demand, it is possible that they increase arterial flow by acting directly on the hepatic vasomotor nerves. If they decreased hepatic arteriolar tone, this would explain in part the hydrocholeresis, since it is known that section of the hepatic nerves has a hydrocholeretic effect (1) and such a change could explain mechanically the reciprocal changes in flow in the hepatic artery and in the superior mesenteric artery and portal vein (1, 18). If this is true, the hepatic vasomotor nerve endings or arterioles are specifically sensitive to unconjugated oxidized cholic acid, because we have found that ketochola-

nates are several times less potent in depressing blood pressure than the cholates (16).

The phenomenon of refractoriness to the intravenous injection of various bile salts which is present initially or develops during the course of an experiment is of much interest. In the course of various studies we have seen 17 cases of refractoriness in 170 dogs. The mechanism concerned is not clear (1).

SUMMARY AND CONCLUSIONS

1. The direct current thermostromuhr (Baldes and Herrick) has been tested on relatively large vessels and found to be accurate to about 15 per cent in a range of flows from 50 to 400 cc. per minute.

2. In 46 experiments on 21 dogs, sodium dehydrocholate given intravenously in 0.5 gram doses produced an increase in hepatic arterial blood flow in 42 instances. The increases ranged from 15 to 218 per cent, averaging 48 per cent. In 16 experiments on 6 dogs, the mixed triketocholanates in 0.25 gram doses produced an increase in hepatic arterial flow in 14 cases. The increase averaged 39 per cent and ranged from 15 to 119 per cent.

3. In 4 experiments on 4 dogs, sodium dehydrocholate given at the rate of 0.5 gram per hour into a mesenteric vein produced an increase in hepatic arterial flow in every case.

4. In two tests, *cinchophen* given intravenously in doses of 50 mgm. per kilo produced an increase of 47 per cent in hepatic arterial flow in both instances.

5. In 27 experiments on 11 dogs, sodium cholate in 0.5 gram doses produced no change in hepatic arterial flow in 11 cases, a decrease in 10, and an increase in 6.

6. Sodium taurocholate in 0.5 gram doses produced a decrease in hepatic arterial flow in 6 of 9 experiments on 5 dogs. Sodium glycocholate produced a decrease in 4 of 5 tests on 5 animals. The average decreases were 16.5 per cent and 13.7 per cent respectively.

7. In 15 experiments on 6 dogs, sodium dehydrocholate produced a decrease in portal venous flow in 8 cases, an initial decrease followed by an increase in 5, and an increase in 1. This salt produced a decrease in superior mesenteric arterial flow in 10 of 14 experiments on 7 dogs.

8. It is suggested that any true hydrocholeretic, as illustrated by sodium dehydrocholate, or the ketocholanic acids, and cinchophen, may increase hepatic arterial flow. On the other hand, substances such as the conjugated cholates which cause only a moderate increase in bile volume output with an increase in total solids without much change in viscosity do not produce this effect.

9. An increase in arterial flow to the liver is not essential for a choleretic response to bile salts.

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TRAINING IN HUMAN MUSCLES WORKING WITH AND WITHOUT BLOOD SUPPLY[1]

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If a muscle is used daily to perform voluntary work its capacity for work will undergo marked enhancement by a process known as muscle training. Little is known of the mechanism by which the power of the muscle increases. It is possible that local changes in the muscle cells themselves may be responsible. Biochemical studies devoted to measuring changes in constituents of muscle as a result of training have not been very illuminating. It is possible that changes in the innervation of the muscle are responsible. This might involve the building of resistance to fatigue in the cells of the motor cortex or the anterior horn or a more economical usage of motor discharge. It is possible that changes in the blood supply may be responsible in increasing the oxygen available and facilitating the removal of metabolites. It seemed reasonable that removal of the influence of the circulation might yield significant information about this problem. Accordingly the training characteristics of muscle working in an ischemic condition (i.e., without blood supply) have been investigated.

METHOD. The experiments required that work be done by a single muscle; that the work be accurately measured; that the muscle be worked to a clearly defined end point each day; that the blood supply be interrupted during work in one muscle while its contralateral fellow worked with its blood supply intact as a control.

The muscle. The extensor digitorum communis muscle was selected for the tests. The arm was pronated on an ergograph with the major flexion crease of the palm at the edge. The fingers hung vertical at rest. The load was applied through a leather strap over the distal half of the middle phalanges of the fore, middle and ring fingers. Thus the fulcrum over which force was applied by the muscle was the metacarpophalangeal joint. The interphalangeal joints were kept extended at all times. This arrangement has the following advantages:

[1] A preliminary report of some of these experiments appeared in the Proceedings of this Journal **129:** 415, 1940.

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1. No muscle other than the extensor of the digits can apply force to the work.

2. The landmarks are so definite that there is little chance that the manner of application of the hand to the ergograph can vary.

3. The muscle is not well trained in ordinary life activities.

The ergograph: A weight ergograph was constructed for the extensor of the fingers. Two automatic counters were so arranged that while one registered the total distance through which the load was moved, the other counted the movements made. Figure 1A shows the ergograph seen from the side with arm in place. Fore, middle and ring fingers are inserted in thong, *q*. The thong is attached by wire to a brass plate 6 by 1 by $\frac{1}{16}$ inch, *n*. This plate is held in position by a steel angle, *o*, which is slotted to receive the plate. A brass pin, *p*, is driven through the plate to act as a stop on the thong side of the angle. At its other end the plate makes connection with a chain, *m*, which passes over a pulley of $2\frac{3}{4}$ inches diameter, *l*. The

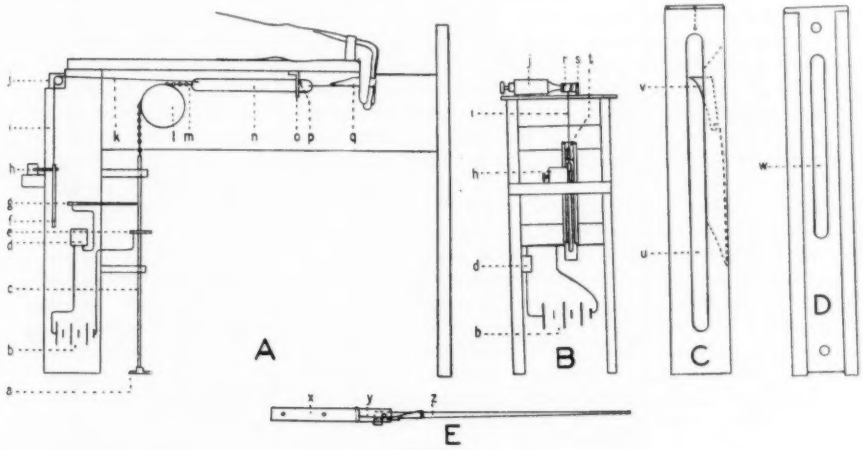

Fig. 1

chain after a vertical downward course of 6 inches attaches to a circular brass bar of $\frac{1}{4}$ inch diameter, *c*. The brass bar reaches to the floor and has threaded to it at its inferior end a circular brass plate $1\frac{1}{2}$ inches in diameter and $\frac{1}{8}$ inch thick which acts as a weight holder, *a*.

Just above the attachment of the chain on the plate, *n*, there is attached a cord, *k*, to operate the meters (*h* and *j* in fig. 1A and B). (Fig. 1B shows the ergograph from the back.) The cord passes to a spindle (*r* in fig. 1B) of 5 cm. circumference and $1\frac{1}{2}$ inches length. After several turns about the spindle the cord is anchored to the spindle. The spindle is united to the shaft of a Veeder reset rotary ratchet counter (no. L244) (*j* in fig. 1A and B) which registers 10 units for each rotation of the spindle. The other side of the spindle is supported by a brass bearing (*s* in fig. 1B) soldered to the plate on which the meter rests. This counter records the distance through which the weights are moved in 0.5 cm. unit.

The spindle also gives anchorage to a second cord (*i* in fig. 1A and B) which runs in opposite direction on the spindle to the first. After several turns this cord runs vertically downward to a brass rectangular bar 6 by 1 by $\frac{1}{8}$ inch (*f* in fig. 1A and in

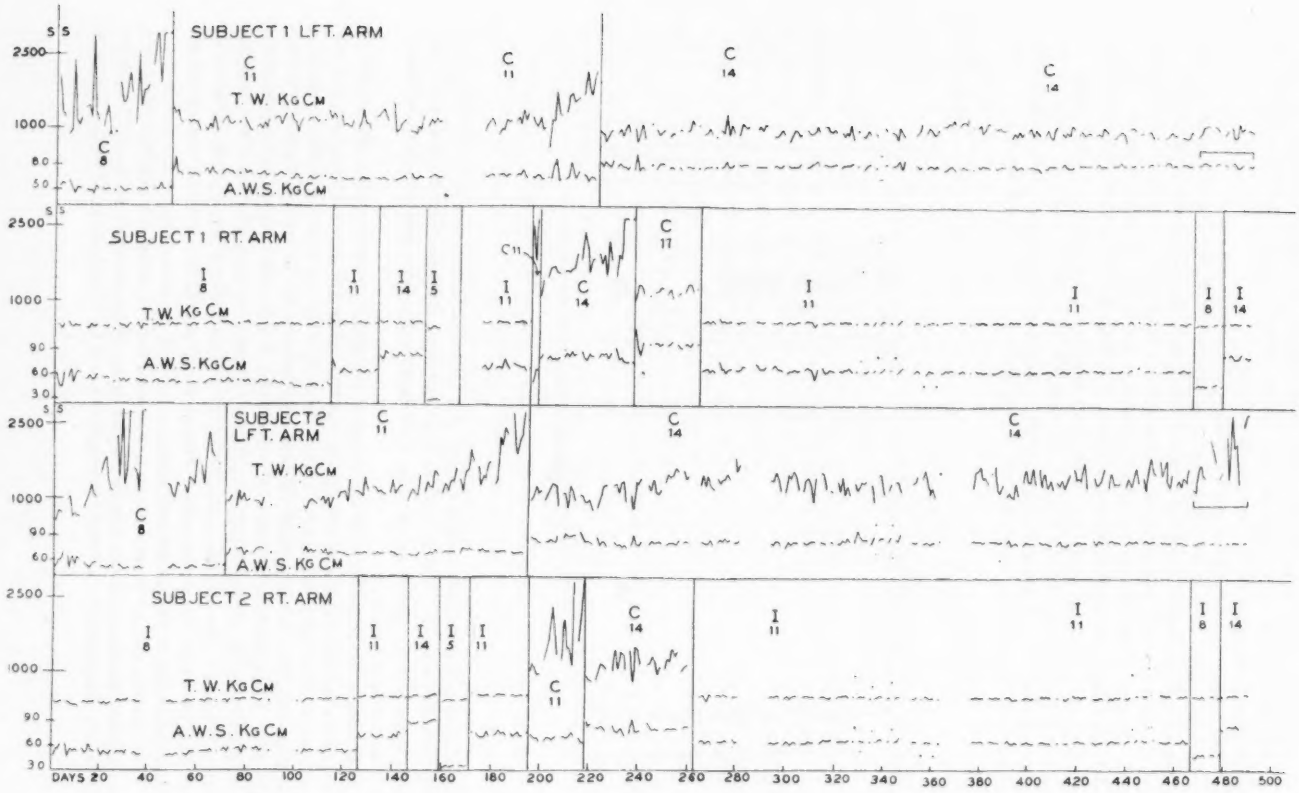
SUBJECT 1 LFT. ARM
T. W. KG CM
A.W.S. KG CM
SUBJECT 1 RT. ARM
T. W. KG CM
A.W.S. KG CM
SUBJECT 2 LFT. ARM
T. W. KG CM
A.W.S. KG CM
SUBJECT 2 RT. ARM
T. W. KG CM
A.W.S. KG CM
2500
1000
90
60
30
DAYS 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500

Fig. 2

detail as *C* in fig. 1). The bar runs in a metal track (*t* in fig. 1B and in detail as *D* in fig. 1). A Veeder reset ratchet single unit counter (no. ZD18) (*h* in fig. 1A and B) is mounted behind this metal track. Figure 1 C and D shows that both the slider bar and the track have a slot (*u* and *w*). The slider bar has a spring cog (*v* in fig. 1C) mounted so that it will move aside if an object approaches it in the slot from below but will hold fast if it is approached from above. The single unit counter has a lever attached to its shaft (see *h* in fig. 1A) which projects through the slots in the slider bar and track. When the machine is at rest the meter lever lies above the spring cog of the slider bar.

When the finger thong is pulled forward the weights are lifted and cord, *k*, is wound off the spindle. This motivates the distance counter. Simultaneously cord, *i*, is wound on the spindle from the slider bar which is thus pulled upward. As the bar moves upward the meter lever catches on the spring cog and is raised tripping the lower counter one unit. After the spring cog has risen about 3 cm., the lever, making an arc, has moved out far enough to slide off the spring cog and to fall back to its normal position. When the tension on the finger thong is released gravity returns the weights to rest, and acting on the slider bar pulls the bar back to rest. As the slider bar moves downward the spring cog is moved aside by the meter lever and again comes to rest below the lever. As the slider bar moves down it unwraps its cord, *i*, from the spindle and at the same time the other cord, *k*, is wrapped onto the spindle. Thus the weight of the slider bar is used as a counterpoise to return the meter cords to resting position after each stroke.

The inherent resistance to motion of the finger thong includes the weight of the load rack (*a* and *c*), the weight of the slider bar, *f*, which moves centimeter for centimeter with the load, and the resistance of the meters (*h* and *j*). This resistance was measured by running a cord from the finger thong over a pulley and vertically downward. Weights hung on this cord overcame the resistance of the ergograph. In construction the weight of the slider bar was so adjusted that the total inherent resistance of the machine was 500 grams. Weights were then made of lead in 300 gram units. Thus loads in 300 grams steps above 500 grams could be obtained.

The distance through which the load was moved was recorded by the counter in ½ cm. units. This implies a maximum error of ¼ cm. in each stroke. Work was obtained as the product of load and distance.

Fig. 2. Plot of daily work of extensor digitorum communis muscle. Condition of muscle during work is indicated by *C* or *I* meaning "with blood supply" or "without blood supply" respectively, and by a numeral—8, 11, 14—meaning the load used in hundreds of grams. Hence *C8* means muscle circulated working on 800 grams load. Rate of work with any given load is kept constant by making 1 stroke per second, each stroke about 6 cm. in length. *T. W. kg. cm.* = total work in kgm. cm. *A. W. S. kg. cm.* = average work per second in kgm. cm. *S S* = steady state performance. Horizontal bracket in last twenty days of top muscle and third from top indicates that during this period the subject was undergoing an added exercise period of 15 minutes per day with a lighter load. Due to the marked reduction necessary certain points on graph are unlabelled: In top and 3rd graphs about 340th day the ischemic ability was tested with 1100 grams load for two days. In second and 4th graphs on three days near 340th day the ability with blood supply was tested with 1400 grams. Soon thereafter the ischemic ability with 800 grams was tested on two separate occasions. When work was done each day the points are connected. When a day elapsed without work, i.e., Sunday, the previous and succeeding points are unconnected.

Definition of fatigue. *Voluntary work without blood supply* produces a different fatigue curve than work with blood supply. The contraction height is well maintained and after a time ischemic pain is felt. Still later the ability to move is rather abruptly lost. Hence in these studies ischemic work was terminated when the subject could no longer lift the weight. Work was done at a rate of 60 contractions per minute.

Voluntary work with blood supply is accompanied by a gradual decline in contraction height. If the contraction height reaches a small enough level the muscle may be capable of continuing work in virtually a steady state. It is thus difficult to establish an end point unless the length of stroke is controlled. If the subject is required to make a minimum stroke of comfortable length the maintenance of a given rate of strokes affords a good end point. The subject continues making strokes of the set length until he can no longer make a stroke in the allotted time interval. Using the extensor digitorum communis adequately weighted at a rate of one contraction per second produces an obvious fatigue under this regime as shown by the marked associated movements, increase of blood pressure and flushing which accompany the last few strokes.

Fixation of stroke length was accomplished by arranging a buzzer to sound when the weights were raised to a given height. The subject was required to ring the buzzer with each stroke. Contacts for the buzzer are seen in figure 1A and B. A circular brass plate (*e* in fig. 1A) 2 inches diameter and $\frac{1}{32}$ inch thick was fixed by a set screw to the weight bearing rod, *c*. A flexible copper wire made connection from the plate to an ordinary door buzzer (*d* in fig. 1A and B). The other pole of the buzzer contacted a dry cell, *b*, whose second pole was connected to a spring lever, *g*, attached to the side board 6 cm. above the contact plate. The spring lever is seen as *E* in figure 1. One piece of brass sheet, *x*, $\frac{1}{2}$ by 3 inches is used as anchor. At its distal end it holds a loose rivet as axle for rotation of the lever, *z*, 6 x 0.010 x $\frac{1}{4}$ inch to $\frac{1}{2}$ inch which is crimped at its lower edge to prevent passage of the lever downward below horizontal. Upward motion of the lever is impeded by a short length of 0.015 inch music wire, *y*, which insures good contact between lever and plate when weights are raised. The effectiveness of this method of limiting the stroke is seen in the lines showing average work per second in figure 2. While the average rate of work with any given load is not constant it is relatively so.

Ischemia of the muscle was created by applying a sphygmomanometer cuff to the arm and inflating it instantaneously from a pressure reservoir of 5 liter capacity at a pressure of 240 mm. Hg. Vascular congestion was avoided by rapid inflation.

Subjects. Twelve healthy male subjects were used. Eleven were graduate students between 22 and 28 years of age, 1 was a mechanician aged 45 (no. 1). All were in good health and all were accustomed to a semi-sedentary life. All had normal blood pressures.

The effect of daily ischemic work on ischemic ability. Nine untrained subjects exercised their extensor digitorum communis muscles to fatigue once each day, 6 days per week. The right arm was worked without blood supply, the left arm with blood supply. A load of 800 grams, a stroke greater than 6.0 cm. and a stroke rate of 60 per minute were employed. Four of the subjects worked for 4 months, the remaining 5 for $2\frac{1}{2}$ to 3 months. Table 1 shows average values of work done by the ischemic muscles at beginning and end of the experiments. A more

complete picture for 2 of the subjects is seen in the first 120 days of the right arm graphs in figure 2. Here one can see the daily variation of total work and of average work per second which reflects the constancy of the stroke distance.

The response of ischemic work capacity to daily work without blood supply was indicated in 6 of the 9 instances by a small increase. Cases 3, 4 and 7 showed almost no change. The others showed increases of from 6 to 25 per cent in the course of 10 to 17 weeks' work. The manner of rise was steady gradual increase in cases 1, 2, 6 and 8, and a sharp rise to near

TABLE 1

Effect of daily ischemic work on ischemic work capacity compared with speed of circulated training in each case

Subject	RIGHT ARM ISCHEMIC DAILY AVERAGE WORK OF EXT. DIGIT. COMMUNIS WITH 800 GRAM LOAD IN KGM.CM.						LEFT ARM CIRCULATED. TRAINING REQUIRED TO PRODUCE STEADY STATE ABILITY IN WEEKS. 800 GRAM LOAD
	In first 3 weeks	In last 3 weeks		Diff.	Tested later after other experiments		
	Kgm.cm.	Weeks	Kgm. cm.		Weeks from start	Kgm. cm.	
				per cent			
1	495	15th–17th	527	+6.5	66th and 67th*	592	4 weeks
2	372	14th–16th	455	+22	66th and 67th*	508	5 weeks
3	485	12th–14th	498	+2.7			6 weeks
4	359	13th–15th	354	−1.4			17 weeks
5	434	9th–11th	541	+25	39th and 40th†	589	11 weeks
6	396	8th–10th	447	+13			Not complete at 11 weeks
7	384	10th–12th	405	+5.5			6 weeks
8	249	7th– 9th	278	+12			Little training at 10 weeks
9	433	7th– 9th	505	+17			5 weeks

* These subject[illegible]daily with various loads throughout this 16 month period.
† This subject wor[illegible]1 weeks then rested 13 weeks then worked 16 weeks.

terminal value within 4 weeks in cases 5 and 9. Subjects 1 and 2 continued working with different loads as shown by figure 2. Subject 5 after a 13 week absence returned to work with other loads. After 66 weeks' work by subject 1 and 2, and 39 weeks' work by subject 5, all 3 worked again without blood supply with an 800 gram load. Table 1 shows that a further increase had occurred. Subject 1 shows a total gain which amounts to about 1.5 per cent per month over the 16 month period.

During the same period described in table 1 the left arms of the 9 subjects were employed in work with blood supply intact. Starting out with 800 grams as the load most of the subjects soon reached steady-state ability as shown in the last column of table 1. There would seem to be little

correlation between speed of circulated training and degree of increase of ischemic work capacity.

Effect of load on ischemic work ability was tested in each of 4 subjects. The data are summarized as averages in table 2. Two of the subjects repeated the test, with the exception of the 500 gram load, 1 year after the original test. The figures show that work capacity was somewhat greater with heavier loads. In subjects 1 and 2 the year's training had apparently eliminated the advantage of the greater load. It is well known that with very light loads the efficiency of the muscle is reduced, since much effort is wasted in process of movement.

TABLE 2

Effect of load on ischemic work ability

Stroke rate of 60 per minute with all loads

SUBJECT	CONDITION	LOAD							
		500 grams		800 grams		1100 grams		1400 grams	
		Trials*	Kgm. cm.	Trials*	Kgm. cm.	Trials*	Kgm. cm.	Trials*	Kgm. cm.
1	After 16 weeks' training	6	471	16	528	16	561	16	577
1	After 50 weeks' training			10	592	10	621	10	614
2	After 16 weeks' training	10	425	11	453	16	498	11	513
2	After 60 weeks' training			10	508	10	517	10	532
3	After 16 weeks' training	4	444	17	505	10	557	17	614
4	After 16 weeks' training	4	343	14	356	14	405	14	430

* Each trial was made on a separate day. The subjects worked only once each day.

The effect of load on ischemic pain accounts for the small number of observations on each subject with the lightest load (500 grams). Such exercise is intensely painful and subjects dislike doing it. The pain is less with greater loads though it comes on after fewer contractions. Finally most subjects showed fatigue without any pain with loads of 1700 grams or heavier. This phenomenon has been previously described by Katz and co-workers (2).

Effect of training with blood supply on ischemic work capacity. This was tested by having the subject work his arm without blood supply once daily until it was thought he was accustomed to the ergograph and his ability was known. In subjects 1 and 2 as shown by figure 2 this test was

made by the right arms after 7 months of daily ischemic work. Both arms of subjects 5, 10, 11 and 12 were tested after 2 to 4 weeks of daily ischemic work practise. Figure 3 shows the plot of the right arm of each subject.

Subjects 1 and 2. (See fig. 2, 170th to 280th day.) The right arms of subjects 1 and 2 were given a 2 month period of circulated training which

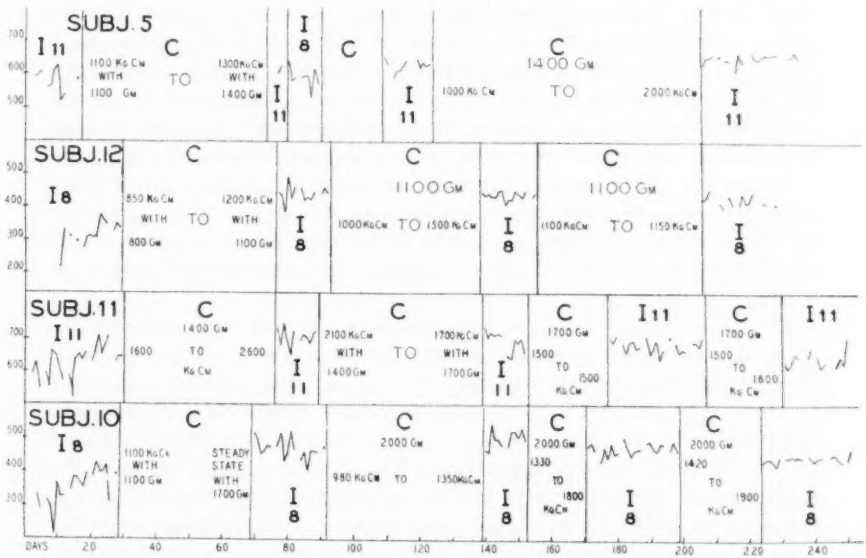

Fig. 3. Effect of circulated training on ischemic work capacity. Plot of daily work of extensor digitorum communis muscles. Total work of muscle without blood supply is plotted, load in hundreds of grams being indicated by the numeral—*I8* means ischemic work load 800 grams. *C* = circulated work to fatigue being done at this time. Work capacity and load at beginning and end of circulated training periods being shown by the numerals to indicate effectiveness of training. Only the results on right arms are shown but left arms were also tested and are described in text. Ordinates show work done in kgm. cm.

very effectively improved their ability to do work with blood supply yet no improvement in ischemic capacity occurred. The graph of subject 1 is especially instructive in this regard since he showed a steady 1.5 per cent per month improvement in ischemic ability from the start to the end. The circulated training did not seem to speed or retard this gradual process.

Subject 10. Both arms ran virtually parallel in ischemic performance, the right arm being capable of about 30 kgm. cm. more than the left. It is to be noted that the first period of circulated work apparently increased

the ischemic work capacity. Although training continued to occur in later bouts of circulated work, the ischemic ability did not increase.

Subject 11. This subject's right arm showed very little gain in ischemic ability as a result of circulated training. His left arm showed no improvement whatever in ischemic ability.

Subject 5. Returned to work after a summer vacation. In view of his previous experience he worked his arms without blood supply for only 2 weeks before beginning circulated training. It will be noted that his ability with 800 grams was tested from the 80th to 90th day of the graph to compare with previous year's ability as shown in table 1. Both right and left arms showed a gain in ischemic capacity throughout the graph which was least after the last period of circulated work although training in circulated ability was greatest in the last period of circulated work.

Subject 12. Both arms of this man ran parallel throughout the experiments with the right arm better by less than 20 kgm. cm. In both arms the ischemic capacity showed marked improvement after the first period of circulated work but there was no further change after later periods of circulated training.

The data obtained show that the gains in ischemic work capacity obtained by daily ischemic work to fatigue for a period of months were small. Most of the trials showed no increase in ischemic work capacity as a result of effective training with blood supply. There were several instances in which increases did occur. Most of these instances were in the first trial of the effect of circulated training. The question arises whether the subjects were completely acclimated to the routine of ischemic work on the ergograph before they started circulated training. The fact that improvement in ischemic performance did not occur after later periods of circulated training suggests that the subjects were not used to the routine.

Any improvements in work capacity of muscles using the ergograph without blood supply may be due to at least two different processes: There might be an increase in skill (learning to time the movements more accurately, to execute them with less waste motion) or there might be an increase of the total energy reservoir of the ischemic muscle. There is evidence that skill continues to improve over a fairly long period of training. Compare the response of ischemic muscle to varying load after 4 months with the response after 14 months of daily work (subjects 1 and 2, table 2). In the earlier trial when movements were probably wasteful and inaccurate the most work could be done with the heavier loads. Ten months later when the movements had become more practised, the advantage of the heavy load had practically disappeared. The comparison does reveal a small improvement in the ischemic performance with all loads as a result of the period of work. Whether greater skill or increase in the energy reservoir is responsible is not obvious.

At all events it is clear that improvement in work capacity with blood supply does not guarantee increase in work without blood supply as is shown by a close examination of the work of the left arm of subject 2 as seen in figure 2. On the 337th and 344th days of the graph the ischemic ability of the arm was tested and found to be 540 and 525 kgm. cm. with 1100 grams' load. Due to the great reduction required for this figure these dots are unlabelled on the graph. At this time the muscle with blood supply averaged about 1350 kgm. cm. with 1400 grams' load. On the 485th and 486th days the muscle was nearing steady state ability with 1400 grams as a result of 15 minutes' daily exercise for 3 weeks with a light load. The ischemic ability was now 535 and 495 kgm. cm. On the 498th day the steady state ability with 1400 grams was well established and yet the ischemic work capacity was but 539 kgm. cm.

Training pattern in muscles working with blood supply. All subjects tested showed the same pattern seen in the graphs of the left arms of subjects 1 and 2 in figure 2. This pattern is characterized by the fact that use of light load of 800 grams gave rapid rise to steady state ability. Changing to 1100 grams fixed the capacity for work at a certain level for 150 days in subject 1, 90 days in subject 2, when both subjects showed a rapid enhancement of ability. The use of a still heavier load (1400 grams) abolished all signs of improvement in subject 1 and only very slight improvement occurred in subject 2 for 175 days. Only when an added exercise period of 15 minutes (900 contractions) of steady-state work with a lighter load was done each day did the muscles of subject 2 become able to carry 1400 grams in steady state. The added exercise did not enable subject 1 to increase his work capacity. This failure may be related to the greater age of subject 1 (45 yrs.). Yet it will be noted that the right arm of this same individual showed steady slow improvement in ischemic capacity (skill or reservoir?). Thus it would seem that if the rate of work is too fast, the muscle working in a relatively anaerobic state, training will fail to occur.

Relationship between the capacities of muscle for different types of work. The usage of muscles in the intact body may be roughly classified into three types: 1. Steady state work capacity, or endurance for aerobic work. 2. Anaerobic work capacity, ability to work without blood supply or so rapidly that the existing power to supply oxygen is negligible compared to the metabolic rate. 3. Absolute power, maximal strength exerted in a single effort. It might be supposed that a strong muscle would be strong in all these usages, a weak one poor in all of them. The data do not support this conception. The three properties seem separate. We have seen that improvement in ability to work with blood supply does not improve ability to work without it. Likewise the fact that the ischemic ability of two muscles is equal does not indicate that their circulated work

capacity will be equal. For example, the ischemic capacity of both arms of subject 2 near the end of the experiment was about 525 kgm. cm. with a load of 1100 grams. The ability with blood supply of the right was not great enough for steady state with 1100 grams whereas that of the left was sufficient to permit steady state with 1400 grams' load.

Similarly, one muscle may have poorer ischemic ability than another though the capacity of the former for training with blood supply may exceed that of the latter. Figure 3 shows that the right arm of subject 10 had an ischemic capacity of only 475 kgm. cm. but was capable of being trained to carry 1700 grams in steady state with blood supply. The right arm of subject 12 with an ischemic capacity of 700 kgm. cm. was incapable of reaching steady state with 1700 grams in the given training period.

Through failure to realize its import no attempt was made at the outset to test the absolute power of the muscles before training. The ergograph offers a convenient method of roughly estimating absolute power. Load can be increased until the unexercised muscle is just able to raise it off the ground. Making these tests near the end of the experiments revealed:

	MAXIMUM LIFT	ENDURANCE
	grams	
Subject 1 Rt.	4350	1100 grams steady state
Subject 1 Lt.	3750	800 grams steady state easily
		1100 grams steady state occasionally
Subject 2 Rt.	4050	1100 grams steady state
Subject 2 Lt.	3200	1400 grams in steady state

Thus the three rough abilities listed above seem distinct. This suggests that they may depend on different processes in the muscle.

The effect of daily ischemic work on the time of onset of ischemic pain. Katz et al. (2) concluded that training must enable the muscle to do more ischemic work before pain appears, from the fact that the right flexor was able to do more work than the left in this circumstance. This was contrary to the conclusion advanced previously by Lewis et al. (3), who considered the time of onset of ischemic pain as invariable. Table 3 shows the time of onset of pain at various periods in the course of the experiments. It is seen that the range was in most subjects quite small and the degree of change with daily ischemic practise small and slow. This would account for Lewis' conclusion that time of onset of pain was constant. In the long run most subjects showed a distinct increase in time of onset, in agreement with Katz' conclusion.

Circulated practise as well as ischemic practise tended to delay pain onset as shown by the table. The effectiveness of the various circulated

work periods in training can be compared with their effect in delaying pain by comparing numbers given for the right arm of each subject in figure 3 with the data in table 3.

A most striking effect of circulated work on ischemic pain cannot be graphically shown. As one works daily without blood supply the final intensity of the pain grows much less. If, then, one works with blood supply for a number of days the pain on returning to ischemic work is very markedly heightened. All subjects volunteered this information. No reason can be advanced to explain this phenomenon but a similar phenomenon may account for the overwhelming character of pain in intermittent claudication and angina pectoris.

TABLE 3

Effect of daily ischemic or circulated work on time of onset of ischemic pain

Each figure given is the average of 10 consecutive daily trials at the stated time

ISCHEMIC PRACTICE											CIRCULATED PRACTICE							
Subject	Load	10th–20th day, Time in sec.			70th–80th day, Time in sec.			470–480th day, Time in sec.			Subject		Load	10 days before circulated training	10 days after circulated training	After 2nd period of circulated training	After 3rd period of circulated training	After 4th period of circulated training
		Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Number	Arm						
1	800	44	51	57	64	67	72	85	89	95	1	Rt.	1100	58	65			
2	800	35	41	50	44	51	65	65	68	75	2	Rt.	1100	52	58			
3	800	45	52	60	50	55	60				5	Rt.	1100	43	43	45		
4	800	31	37	52	45	52	58				5	Lt.	1100	36	36	45		
5	800	33	36	48	40	56	60				10	Rt.	800	50	55	54	63	59
6	800	43	49	59	No pain						10	Lt.	800	49	55	55	63	
7	800	32	39	50	35	39	48				11	Rt.	1100	48	57	59	56	60
8	800	28	30	35	30	33	35				11	Lt.	1100	45	56	61	64	
9	800	43	52	60	40	64	73				12	Rt.	800	23	46	45	50	
											12	Lt.	800	23	43	65	56	

Validity of method. The literature contains many contributions which criticize and evaluate the various types of ergograph. Limitation of space prevents complete discussion of these criticisms. The most important refer to: 1. Variation of leverage as the stroke progresses results in a different amount of work per centimeter in short strokes than in long ones. This means that the meter would measure a different fraction of the work done by the muscle in a long stroke than in a short one. This objection does not apply to the present method since each stroke is of uniform length and thus the fraction of the work measured should be uniform. 2. Measuring accurately the work done with a weight ergograph is difficult due to the fact that weights have inertia. It is not claimed that the present method measures all the work done by the muscle, nor that it measures

all the work the muscle can do. It is thought that a constant fraction of the work done is measured, and that by working the muscle daily to a uniform end-point of fatigue a constant fraction of all the work the muscle can do is obtained. The fraction of the work done which appears in our figures must be a large one. 3. The objection can be raised that experiments whose end point depends upon human judgment are apt to be in error. In the type of experiment reported here the subject stopped work in ischemic exercise when he could no longer raise the load to ring the buzzer. How do we know he really could not raise the load? As fatigue sets in greater and greater effort was required to raise the load. Associated movements, grimaces, movements of legs, of free arm and of the trunk increased with the effort. The blood pressure gradually mounted. Actual failure of power came on suddenly and could almost be predicted by an accustomed observer. In the work with blood supply the end point was inability to make the full contraction in the allotted time of 1 second. When the subject was fresh, the contractions were well spaced, he paused in rest between them. As he tired his efforts grew more and more continuous. In his attempt to keep up with the rhythm he worked harder. Associated movements were even more marked here than with the ischemic work since the end was less sudden. The degree of impotence of the muscle so worked in the subject well accustomed to the routine was remarkable. If immediately after he stopped work he was asked to raise the weight again in a leisurely fashion it was all he could do to raise it and sound the buzzer in 4 or 5 seconds of continuous effort. When the subject worked daily with the same load, it felt heavy to him on some occasions, light on others. Hence if without his knowledge one gave him a load different by 300 grams he could not always recognize the difference. Thus one could readily check on his honesty. Such tests, watching associated movements, and occasionally taking blood pressure at the end of work were convincing evidence of the honesty of the subjects. The actual arterial blood pressure rise with this effort in accustomed subjects varied from 40 to 80 mm. Hg. No tension above 200 mm. Hg was ever noted.

Discussion. The ischemic work capacity of muscle showed no sizable increase as a result of either ischemic or circulated practise. This suggested that improvement in work capacity of circulated muscle as a result of training must depend on changes in circulatory and central nervous systems rather than on changes in the muscle cells themselves. The importance of local vascular changes in muscle training has not been emphasized. However, there are two logical mechanisms by which these changes might occur. A greater number of capillaries per muscle fiber might be developed, and a quicker and more effective increase in flow through existing capillaries by improvement in reflex control of vessels would be useful. The former mechanism is supported by the data of

Petren, Sjöstrand and Sylvèn (4) on capillary counts in gastrocnemius muscles of trained and untrained guinea pigs. They showed increases in number of capillaries per muscle fiber as great as 40 per cent above the control levels in trained animals. These changes were slower to occur and smaller in older than in younger animals.

The conception that muscle training depends on vascular changes rather than on changes within the muscle fiber is quite logical. Improvement in aerobic endurance is the principal performance change in trained muscle which has been objectively demonstrated, i.e., steady state work. The limiting factor in this kind of work is probably the ability of the blood to supply oxygen to the muscle fiber. An increase in the number of capillaries per fiber would increase the oxygen supply.

Increase in size of muscle is commonly the most emphasized effect of training. It is generally accepted that the cause of this increase in size is due to hypertrophy of the individual fiber. It is unlikely that this process would increase the aerobic endurance of muscle since it involves reduction of diffusional surface area per unit volume of muscle fiber and would thus reduce the oxygen supply. In fact, there is evidence that hypertrophy is not a uniform concomitant of muscle training. Dawson (1) states that size of muscle remains unchanged with the slow steady exercise that produces good training; only in rapid short exercise (involving sudden strain) does hypertrophy occur. The long distance runner in perfect trim shows small muscles; the weight-lifter becomes "muscle-bound."

Petren et al. (4) in their experiments on capillary counts found no difference in the size of muscle fibers from trained and control guinea pigs. Another indication that training and hypertrophy are separate processes is seen by comparing the course of the two processes. Siebert (5) showed that when rats were worked at a particular rate a certain amount of hypertrophy would occur. Persistence in work at this speed would occasion no further hypertrophy; only by increasing the speed of work could hypertrophy be continued. In contrast to this is the pattern of training reported here in muscles working with blood supply in which raising the rate of work resulted in a prolonged period of failure to gain endurance. Only when there was instituted a long exercise period with lighter load did greater endurance with the heavier load appear. Thus increase of endurance and hypertrophy are not simultaneous and may indeed be unrelated processes. There is a possibility that hypertrophy may be a by-product of training, perhaps even a noxious by-product.

Present biochemical evidence suggests that the energy release mechanisms of aerobic and anaerobic work in muscle may be separate. It is therefore reasonable that ischemic work capacity does not improve with increase in circulated work capacity.

The failure of ischemic practise to produce training, and the slow rate

of gain of endurance with rapid rates of work indicates that endurance for work with heavier loads is gained best by much practise with lighter loads.

SUMMARY

1. A weight ergograph adapted for the measurement of the work of human extensor digitorum communis muscle is described, whereby the total distance of vertical lift, the number and uniformity of individual movements made, and the stroke length are accurately gauged.

2. The muscles were voluntarily worked to fatigue once each day. The right arm was worked without blood supply, the left with blood supply. Training patterns without blood supply showed very small and slow gains in total work done amounting to an increase of, at most, 30 per cent in 16 months. The work ability of muscle working without blood supply was not significantly increased by effective training with blood supply. The work capacity of muscle without blood supply was somewhat greater with heavier than with lighter loads, but this difference was reduced by prolonged use of the machine and the development of skill. The time of onset of ischemic pain was delayed only very slowly by daily work without blood supply. The intensity of ischemic pain was markedly reduced in the process. An interim of daily work with blood supply reintensified the ischemic pain on later trials without blood supply.

3. The training pattern of muscles working with blood supply showed rapid assumption of steady state ability with light load. Shifting to a load 300 grams heavier resulted in a long period of static work capacity with finally a rapid increase in work ability. A load 300 grams heavier still abolished all signs of training for 9 months. Yet, a daily exercise period of 15 minutes with lighter load served to increase the work capacity with the heavier load.

4. It is concluded that the anaerobic energy release mechanism in muscle undergoes little change as a result of training. Training would seem to be largely a phenomenon of improvement in nervous direction and vascular supply.

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OVULATION INDUCED IN MICE BY SINGLE INJECTIONS OF FOLLUTEIN[1] OR UNTREATED HUMAN PREGNANCY URINE[2]

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Since Friedman (1) showed that ovulation could be induced in the rabbit by a single intravenous injection of the urine of pregnant women, numerous investigators have experimented with other animals to determine whether they will similarly respond to gonadotropins in pregnancy urine (PU).

In mice, ovulation induced by PU substances has been reported, but this seems to occur only after the animals have been injected for several days. This is particularly true of the immature mice. Hill (2) observed ovulations in 21-day old mice after four daily injections and in 14-day old animals treated for six days. Nelson and Overholser (3) reported ovulations in mice 20 to 22 days old, after injecting Antuitrin-S or pregnant mare serum twice daily for three days. Osborn (4) induced ovulation in dwarf mice, which are usually sterile, after first stimulating the ovaries with FSH twice daily for four days, then injecting LH.

Since these other investigators were concerned with the results of several injections, this experiment was undertaken to determine the possibility of inducing ovulation in mature, non-pregnant mice, pregnant mice and late immatures by a single subcutaneous injection of either the commercial extract, Follutein, or untreated human pregnancy urine.

PROCEDURE. The albino mice used in these experiments were from the Rockland strain. Each animal received only one subcutaneous injection. At first only mature unmated females were injected with Follutein. Later, when it was found that Follutein regularly induced ovulation, a smaller number of mature unmated, mated, and also immature female mice were similarly injected with 0.5 cc. of untreated urine from human pregnancies of approximately two and one-half months' duration. This amount of urine, chosen empirically, gave consistent results, and was easily tolerated. These animals were autopsied 18 to 20 hours after the injection. No attempt was made to find minimal ovulation doses.

[1] We are indebted to Doctors J. A. Morrell and G. A. Harrop of E. R. Squibb and Sons for a generous supply of Follutein.

[2] The human pregnancy urine was obtained through the coöperation of Dr. R. O. Hitchcock, Alfred, New York, and Dr. H. S. Brasted, Hornell, New York.

Each half of the reproductive tract was removed intact, washed with normal saline solution, and placed in a deep-well slide for inspection under a dissecting microscope. The ampulla of the oviduct was then carefully examined by reflected light. Ovulation was plainly indicated by the appearance of the ampulla, which was distended by fluid and granulosa cells accompanying the eggs. The extent of ampullar distention was, of course, proportional to the number of eggs and the amount of fluid present.

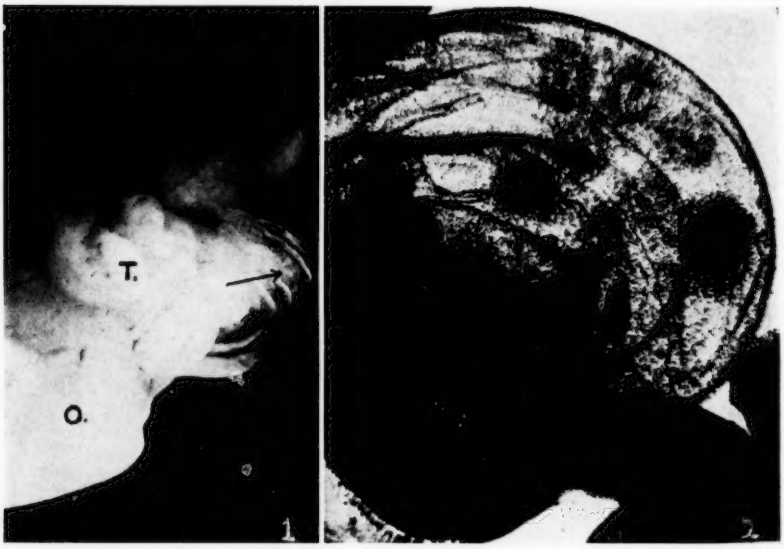

Fig. 1. Photomicrograph of a mouse ovary, *O*, uterus, *U*, and fallopian tube, *T*, as they appear under the microscope before either removal of the ovary or straightening of the tightly coiled oviduct. Arrow points to ova and granulosa cells within the distended ampulla. The photomicrograph, × 12, was taken by means of reflected light.

Fig. 2. Photomicrograph of the ampulla of a mouse oviduct. Flattening the distended ampulla by pressure on the cover glass has separated the cumulus cell mass into component ova with cumulus cells. Eight ova can be seen within the thin ampullar walls. Photomicrograph taken with transmitted light.

Two methods have been used for determining the contents of an ampulla. First, when it was desired to determine only the presence and not the number of ova, the tube was exposed to the light of a good microscope lamp directed down onto the stage at about a 45° angle while the ovary and folds of the mesosalpinx were manipulated with fine-pointed Boley forceps. In this light, a mass of granulosa cells could be distinguished within the thinned ampullar wall (fig. 1) and, usually, individual eggs could be seen as grayish-white spheres surrounded by halos of cumulus cells.

The second method, hereinafter referred to as the pressure method, took a little more time but was the better procedure for counting the eggs. The tract in a deep-well slide under the dissecting microscope was so exposed that the ovary could be cut away from the oviduct. Then the coiled oviduct was separated from the uterus, together with a small portion of that organ which was used for handling the oviduct during later operations. The mesosalpinx, which normally holds the ovary close to the anterior end of the uterus and helps to make a coil of the oviduct, was severed in one or two places to allow a partial straightening of this tube. For the observation of the tract during this procedure, a substage light is

TABLE 1

Ovulation induced with follutein, in mature non-pregnant mice

ANIMAL NUMBER	AUTOPSY HOURS AFTER INJECTION	TUBAL OVA WITH GRANULOSA CELLS	ANIMAL NUMBER	AUTOPSY HOURS AFTER INJECTION	TUBAL OVA WITH GRANULOSA CELLS
Injection: 300 rat units			Injection: 200 rat units		
1	24	7	1	29	9
2	24	9	2	28	6
3	23	6	3	28	3
4	23	10	4	27	No ovulation
5	20	8	5	24	12
6	16	5	6	20	3
			7	18	8
			8	14	4
			9	13	No ovulation
			10	12	No ovulation
			11	11	No ovulation
			12	11	No ovulation
			13	10	8
			14	9	No ovulation

sometimes superior to reflected light. However, individual eggs in the ampulla could always be seen much more clearly if a microscope cover glass were lowered onto the partially straightened oviduct. Gentle pressure applied to the cover glass flattened and squeezed aside the granulosa cells sufficiently to show each egg. Care was taken not to rupture the swollen ampulla. Figure 2 is a photomicrograph of a typically distended ampulla which was flattened by this pressure method. The egg cluster has been broken up so that at least 8 ova with halos of cumulus cells can be counted.

RESULTS. A. *Effect of Follutein on normal, non-pregnant mice.* Mature mice were injected once only with 200 or 300 R.U. of Follutein, and autopsies were performed 9 to 29 hours later. An examination of table 1 shows that an injection of 200 R.U. is apparently as effective in causing ovulation as is the higher dosage. Fourteen (70 per cent) of the 20 animals

had ovulated, and of those autopsied 14 or more hours after the injection, all but 1 (approximately 93 per cent) had eggs with granulosa cells in the ampulla. Of the six autopsied earlier than 14 hours after the injection, only 1 had ovulated. This was mouse 25, with eggs 10 hours after the injection, but this animal may have been in estrus and about to ovulate spontaneously.

TABLE 2

Induced ovulation with 0.5 cc. untreated human pregnancy urine

ANIMAL NUMBER	TUBAL OVA		TOTAL	COMMENTS
	Right	Left		
Mature unmated female mice				
1	3	6	9	
2	5	5	10	
3	9	6	15	
4	5	13	18	
5	4	4	8	
6	5	7	12	
7	5	5	10	
8	6	2	8	
Mated female mice				
1	0	0	0	1-celled ova in oviducts without granulosa cells
2	0	0	0	4-celled ova in lower oviduct
3	0	0	0	2-celled ova in oviduct
4	6	12	18	4 implants about 18th day of pregnancy
5	2	8	10	8 small implants 7 to 8 days
6	8	4	12	6 small implants 7 to 8 days
7	9	7	16	12 implants 12 to 13 days
Immature female mice: Vaginal orifice closed				
1	0	0	0	11.5 grams
2	0	0	0	12.0 grams
3	0	0	0	12.8 grams
4	1	6	7	14.0 grams
5	2	2	4	14.4 grams
6	13	8	21	15.0 grams

These results are particularly interesting because they show that ovulation can be expected 10 to 15 hours after the PU extract injection.

B. *Results following a single injection of 0.5 cc. pregnancy urine.* 1. *With mature non-pregnant female mice.* Ovulation was induced in each of the 7 animals of this series. The total number of ova in both ampullae varied from 8 to 18 with an average of 12 eggs, which is more than the usual number in a normal ovulation. (See table 2.)

2. *With pregnant female mice.* The results in this group are of interest for two reasons. First, in none of the 3 animals with early embryos still unimplanted, had induced ovulation taken place. No conclusions could be drawn from so small a number of animals, but it is possible that there is a definite refractory period following normal ovulation, or else that the dosage is not correct to induce ovulation under these conditions.

In the second place, however, ovulation was readily induced after implantation of embryos had taken place. Animals with small beaded uteri (7 to 8 days *post coitum*) responded to the injections with 10 to 12 eggs each. One animal in late pregnancy (16 to 18 days) was found with a total of 18 eggs in the ampullae. These results show that these ovulations were not cyclic but were induced by the injected pregnancy urine.

3. *With late immature female mice with unopened vaginal orifices.* It must be admitted that this phase of the experiment was undertaken with little expectation of positive results. However, the first animal autopsied (weight 14 grams, approximately 35 days old) had 7 eggs in the ampullae. The animals weighing 14 grams or more ovulated, but none of those weighing less than 13 grams had ovulated. (See table 2.)

Although all of the animals in this series were late immatures, in no instance had the vaginal orifice opened at the time of autopsy.

Discussion. One may now wonder whether some of the earlier experiments of others on the luteinization of mouse and rat ovaries might not have been accompanied by unobserved, induced ovulations. This could have occurred when the animals were injected for several days, and when the autopsies were delayed until after the eggs had had time to disintegrate. On the other hand, some of the injections of other investigators were quite likely to have been excessive dosages that resulted in increased luteinization which, in turn, inhibited ovulation. However, sub-threshold amounts injected for several days are also known to cause luteinization without ovulation. Friedman (1) obtained such results in the rabbit with sub-minimal doses of prolan. These differences in results serve to emphasize the importance of time-dosage factors.

The experiments reported here also show that ovulations probably occur earlier than suspected. During the first 24 hours after the supposed time of ovulation, the ampulla should be carefully examined for eggs surrounded by granulosa cells, but later than this the eggs may be denuded of these sticky cells and found in lower regions of the tubes or in the uteri. In fixing the time of induced ovulation in these mice at 10 to 15 hours after a single subcutaneous injection, we find that these results are comparable to those of Friedman (1), who found ovulation in rabbits 10 to 15 hours after one intravenous injection of PU. Since ovulation-time after subcutaneous injections could be expected to be somewhat longer than by

the intravenous route, we are at present autopsying our mice 16 to 18 hours after injections.

At first it might appear that normal female mice selected at random were not suitable animals for this experiment, since their estrous cycles are usually only 4 to 5 days, and some of the mice, selected by random, might be expected to ovulate within 15 hours after the injection. However, the induction of ovulation in pregnant and immature mice indicates that the ovulations following PU extracts in mature unmated animals are not, to any great extent, the result of fortuitous timing of the injections.

SUMMARY

Single subcutaneous injections of the urine extract, Follutein (Squibb), induce ovulation within 15 hours in mature, non-pregnant female mice.

Similar injections of 0.5 cc. of untreated human pregnancy urine likewise induce ovulation in mature, non-pregnant female mice, and also in pregnant animals with implanted embryos, and in late immature mice with unopened vaginal orifices.

Animals in early pregnancy, with fertilized ova still in the oviducts, appeared to be refractory to this amount of pregnancy urine, and immature females weighing less than 13 grams also failed to ovulate.

A method for counting the ova within the oviduct is presented, and the general appearance of the ampulla after an induced ovulation is shown in photomicrographs.

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RELATIONSHIP OF THE PLASMA VOLUME AND THE CELL PLASMA RATIO TO THE TOTAL RED CELL VOLUME

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The proportion of cells and plasma in blood removed from the body is determined by the hematocrit. As the hematocrit reading of blood taken from the arteries, capillaries and veins of the extremities is approximately the same, and as in dogs blood from the viscera with the exception of the spleen has the same hematocrit reading as blood from the extremities (1, 2), many investigators have assumed that by the use of the hematocrit the actual cell plasma ratio of the circulating blood can be obtained. On the basis of this assumption, many workers have calculated the red cell volume from the plasma volume and hematocrit reading and have estimated changes in plasma volume from changes in the cell plasma ratio or hemoglobin concentration. Smith, Arnold and Whipple (2), however, believed that the hematocrit reading did not give the true cell plasma ratio of the blood as a whole. They demonstrated that the red cell volume as determined by the CO method and the Welker method was lower than the red cell volume determined from the plasma volume (dye method) and the hematocrit reading. Since the CO and Welker methods for measuring the red cell volume did not depend on the hematocrit reading, they concluded that these methods gave the correct red cell volume, and that the red cell volume calculated from the plasma volume and hematocrit reading was falsely high because the hematocrit did not give the correct cell plasma ratio of the entire blood. It was further shown (3) that when the hematocrit reading was lowered by bleeding, the measured red cell volume (from the plasma volume and hematocrit reading) did not agree with the red cell volume predicted on the basis of the volume of red cells removed.

In the study of the circulation, it is essential to know whether the hematocrit reading represents the true cell plasma ratio of the circulating blood. If it does not, in what direction is the error and what is its magnitude? With an accurate method for measuring the plasma volume, this information can be obtained by determining the plasma volume and hematocrit reading before and after removal of a known quantity of red cells. The

red cell volume is calculated from the plasma volume and hematocrit reading. If the red cell volume before hemorrhage is equal to the red cell volume after hemorrhage, plus the volume of the red cells removed, the hematocrit gives the correct cell plasma ratio of the entire blood; if the red cell volume before hemorrhage is greater than the red cell volume after hemorrhage plus the volume of the red cells removed, the hematocrit reading does not represent the cell plasma ratio throughout all of the blood. In normal human subjects the red cell volume 72 hours after venesection always appeared lower than the red cell volume predicted from the prehemorrhage red cell volume and the volume of red cells removed (4). The changes in hematocrit readings were, however, relatively small. This paper reports the results of similar experiments in dogs in which a marked drop in hematocrit reading was produced by massive bleeding.

METHOD. Six complete experiments were performed on 4 unanesthetized, splenectomized dogs. The spleens were removed because it has been shown that in dogs under certain conditions the spleen discharges blood rich in cells into the general circulation (5). The circulation in splenectomized dogs resembles that of normal human subjects because in man there are no significant blood reservoirs (6). The plasma volume, hematocrit reading, and hemoglobin concentration were determined on two successive days. The red cell volume was calculated from the plasma volume and hematocrit reading, the total circulating hemoglobin from the total blood volume and the hemoglobin concentration in grams per cent. If the values for these determinations checked within 5 per cent on two successive days, bleeding was begun in the next 24 hours. A 2 per cent solution of sodium citrate was used as an anticoagulant. The hematocrit reading and hemoglobin concentration of the blood and citrate mixture were determined, and the total volume of red cells and the total grams of hemoglobin removed were calculated. At the end of each bleeding the plasma of the blood removed was separated from the red cells and returned to the dog. Within 48 hours from the time of the first bleeding the plasma volume, the hematocrit reading, and hemoglobin concentration were again determined.

The plasma volume was measured by the dye method of Gregersen, Gibson and Stead (7) as adapted to the Evelyn micro-colorimeter (8). The hematocrit was determined by the method previously described, using 1.6 per cent solution of potassium oxalate as the anticoagulant (9). The total blood volume was calculated from the plasma volume and hematocrit reading. The hemoglobin concentration in grams per 100 cc. of blood was determined by the method of Evelyn (10). The jugular vein was used for injecting the dye and for taking blood samples. Stasis was carefully avoided.

RESULTS. The results were essentially the same in all 6 experiments (table 1). The prehemorrhage red cell volume (calculated from the

plasma volume and hematocrit reading) was from 21 to 34 per cent greater than the red blood cell volume after hemorrhage (calculated from the plasma volume and hematocrit reading) plus the volume of red cells removed by bleeding. Obviously, if the red cell volume calculated from the plasma volume and hematocrit reading was an accurate measurement, these two values would have been identical. The estimation of the prehemorrhage red cell volume from the plasma volume and hematocrit reading is not as accurate as is the calculation of the prehemorrhage red cell volume by adding the volume of red cells removed by venesection to the red cell volume after hemorrhage. In the former the entire measurement is an indirect one based on the assumption that the hematocrit actually

TABLE 1

Typical experiment demonstrating error in calculation of red cell volume from plasma volume and hematocrit reading

DATE	PLASMA VOLUME	RED CELL VOLUME	HEMATOCRIT READING	HEMOGLOBIN	TOTAL CIRCULATING HEMOGLOBIN	NUMBER OF TIMES BLED	RED CELLS REMOVED	HEMOGLOBIN REMOVED	VOLUME OF RED CELLS REMOVED PLUS RED CELL VOLUME AFTER BLEEDING	ESTIMATED PER CENT ERROR IN PREHEMORRHAGE CELL VOLUME	HEMOGLOBIN REMOVED PLUS TOTAL CIRCULATING HEMOGLOBIN AFTER BLEEDING	ESTIMATED PER CENT ERROR IN PREHEMORRHAGE TOTAL CIRCULATING HEMOGLOBIN
	cc.	*cc.*		*grams/ 100 cc.*	*grams*		*cc.*	*grams*	*cc.*		*grams*	
7–10–40	1,140	1,390	55.0	18.3	463		25	8				
7–11–40						3	784	274				
7–12–40	1,610	230	12.7	4.2	77				1,039	34	359	29
8–27–40*	1,280	1,180	48.0	15.4	379		25	8				
8–28–40						3	702	233				
8–29–40	1,610	200	10.9	3.4	62				927	27	303	25

* The dog was not bled from 7–12–40 to 8–27–40.

determines the cell plasma ratio of all the blood in the body; in the latter 75 per cent of the value for the red cell volume is determined by accurate measurements outside the body, and only 25 per cent is dependent upon the indirect measurement involving the assumption that the hematocrit determined the cell plasma ratio of all the blood in the body. The true red cell volume before hemorrhage is, therefore, approximated by adding to the volume of the red cells removed by venesection the red cell volume after venesection (from the plasma volume and hematocrit reading). The value obtained for the prehemorrhage red cell volume as determined from the hematocrit reading and plasma volume is falsely high. The total circulating hemoglobin as estimated from the total blood volume (from plasma volume and hematocrit reading) is also falsely high (table 1).

The falsely high red cell volume as determined from the plasma volume

and hematocrit reading can be explained in three ways: 1, the plasma volume technique gives a falsely high plasma volume; 2, the hematocrit does not determine the true cell plasma ratio of the blood removed, because plasma is trapped among the packed red cells; 3, the cell plasma ratio of the circulating blood as a whole is lower than the hematocrit reading of blood drawn from the body.

If dye were lost into the tissue fluids and returned into the blood stream during the time of the determination, the value for the plasma volume would be too high. When the plasma volume is calculated from the slope of the disappearance curve of the dye, loss of dye into the extracellular fluids would not affect the determination unless large quantities of dye-stained lymph enter the blood stream during the determination. The lymph flow from the thoracic duct of fasted resting dogs (11) is so slow that the entry of dye-stained lymph could not cause an appreciable error in the plasma volume technique.

By calculating indirectly the cell plasma ratio of blood drawn into a flask, it was possible to demonstrate that the hematocrit reading, as determined in the routine manner, represents the true cell plasma ratio of the blood removed from the body and that an appreciable amount of plasma is not trapped among the red cells. Approximately 100 cc. of blood were withdrawn from the antecubital vein of a normal subject in a syringe coated with vaseline. The blood was placed in a paraffin flask containing 0.2 cc. of 0.1 per cent solution of Evans blue and the flask was rotated for 5 minutes. At intervals blood was placed in sample tubes and allowed to clot. The total quantity of blood was determined from the weight of the blood removed and the specific gravity. The cell plasma ratio was determined *a*, from the hematocrit reading in the usual manner, using 1.6 per cent solution of potassium oxalate as the anticoagulant, and *b*, from the plasma volume as calculated from the dye concentration and the total quantity of blood. The dye concentration in the serum of the samples of clotted blood was measured by the microcolorimeter in the usual manner. Two experiments were performed using blood drawn from two different subjects. In each case the cell plasma ratio, as calculated by the dye method, agreed within 3 per cent with that determined by the hematocrit (table 2). Using a slightly different technique, Gregersen and Schiro obtained similar results (12).

The third explanation is, therefore, the correct one. The cell plasma ratio of blood circulating in the entire body is lower than that of blood removed from the body. The red cell volume after hemorrhage plus the volume of red cells removed is, therefore, less than the red cell volume determined before hemorrhage, because the hematocrit reading does not measure the cell plasma ratio of the entire blood. From these experiments it appears that when the hematocrit reading is between 40 and 50 the red

cell volume as measured from the plasma volume and hematocrit reading is about 25 per cent higher than the true red cell volume. In calculating the total volume with a hematocrit reading of 50 this error is reduced by about one-half, making the measured total volume about 11 per cent higher than the true total volume.

When the hematocrit reading is between 40 and 50, the total circulating hemoglobin as measured from the total blood volume and the concentration of hemoglobin in grams per cent is approximately 25 per cent higher than the true total circulating hemoglobin. Both of the values used in the calculation, namely, the total blood volume and the hemoglobin concentration, are falsely high. The fact that the error in total circulating hemoglobin parallels the error in the total red cell volume indicates that changes in the size of the individual cells played no rôle in these experiments.

TABLE 2

Calculation of the volume of cells in blood drawn into a flask from the volume of blood and the volume of plasma

A known amount of dye was added to the blood and the plasma volume was calculated from the concentration of dye in the serum.

SUBJECT	VOLUME OF BLOOD	VOLUME OF PLASMA AS CALCULATED FROM THE CONCENTRATION OF DYE	PREDICTED HEMATOCRIT READING FROM VOLUME OF BLOOD AND VOLUME OF PLASMA	HEMATOCRIT READING
	cc.	*cc.*		
E. S.	93	52.5	43.5	45.0
R. E.	100	54.5	45.5	46.3

Discussion. These observations support the conclusions of Smith, Arnold and Whipple (2) that the red cell volume cannot be measured from the plasma volume and hematocrit reading. By a comparison with the CO and Welker methods, they concluded that with a hematocrit reading of 50 the value obtained for the red cell volume from the plasma volume and hematocrit reading was approximately 20 per cent higher than the actual red cell volume. Although a different method was used in this study, than that used by Smith, Arnold and Whipple, the results agree fairly closely as to the magnitude of the error when the red cell volume is calculated from the plasma volume and hematocrit reading.

The physical characteristics of blood flowing in small tubes satisfactorily explain the apparently paradoxical phenomenon that although the blood removed from artery, capillary and vein has the same cell plasma ratio, this is not the cell plasma ratio of the total circulating blood. Fåhraeus (13) compared the hematocrit reading of blood flowing through a fine tube (obtained by sealing the ends of the capillary tube and centrifuging

it) with the hematocrit reading of the blood that had flowed out of the tube. The cell plasma ratio of the blood dripping from the tube was higher than that of the blood within the tube. He concluded that blood streaming through narrow tubes is relatively much richer in plasma and poorer in corpuscles than the same blood streaming through larger tubes. He points out that it is impossible to obtain the hematocrit reading of blood flowing in a narrow vessel by collecting blood from the cut end of the vessel. The axial portion of the stream, which is rich in red cells, flows out of the cut end while the slower moving peripheral layer of plasma remains in the vessel.

SUMMARY AND CONCLUSIONS

1. Six bleeding experiments were performed on four splenectomized, unanesthetized dogs. The plasma volume and hematocrit reading were determined before hemorrhage. After the removal of a known quantity of red cells, the plasma volume and hematocrit reading were again determined. The red cell volume before hemorrhage was calculated *a*, from the prehemorrhage plasma volume and hematocrit reading, and *b*, from the sum of the post-hemorrhage red cell volume (plasma volume and hematocrit reading) and the volume of red cells removed. Evidence is presented to show that the calculation of the red cell volume from the sum of the post-hemorrhage red cell volume and volume of red cells removed represents approximately the true red cell volume.

2. When the hematocrit reading is approximately 50, the red cell volume as calculated from the plasma volume and hematocrit reading is approximately 25 per cent higher than the true red cell volume. This error occurs because the cell plasma ratio of blood removed from the body is higher than that of the entire circulating blood.

3. The hemoglobin concentration of blood removed from the body is higher than the hemoglobin concentration of the entire circulating blood. When the hematocrit reading is approximately 50, the total circulating hemoglobin is approximately 25 per cent higher than the true total circulating hemoglobin.

4. As the hemoglobin concentration and the cell plasma ratio of blood removed from the body are not representative of all the blood in the vascular system, it is not possible to accurately quantitate changes in plasma volume from changes in either the hemoglobin concentration or hematocrit reading.

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DISTRIBUTION OF WATER AND ELECTROLYTES IN EXPERIMENTAL DIABETES MELLITUS[1]

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Although the composition of the serum in experimental diabetes mellitus and in patients suffering with the disease has been studied at length, comparatively few data are available regarding the total quantities of the serum components or of the distribution of body water present in the various stages of this disease. Quantitative chemical analyses are limited to estimations of concentration. To determine the total amount of a component in the serum the additional measurement of serum volume is obviously necessary.

The few measurements of serum volume reported in cases of diabetes mellitus suggest that in the milder group the serum volume is not altered in any special manner but perhaps is subjected to greater variations than is observed in normal individuals. In the more severe group, and especially in individuals with ketosis, the measurement of serum volume by the more recent dye methods is practically precluded because of the undesirability of withholding treatment during the length of time required for making the measurement (i.e., 1½ to 2 hrs.). Chang, Harrop and Schaub (1) employing the carbon monoxide method for measuring blood volume found that the total plasma volumes may be diminished as much as 24 per cent when compared to the values obtained after recovery.

Under certain conditions relative changes in the amounts of given components circulating in the serum may be estimated without the measurement of serum volume. If it be assumed that during a period of observation the amounts of serum protein or serum solids remain unchanged and that differences in their respective concentrations are due to loss or gain of serum water, then the changes in the quantities of serum components may be calculated from these differences. Utilizing this indirect method we have reported from this laboratory (2) (3) changes in the quantities of various serum constituents following the ingestion of glucose in diabetic individuals and following the administration of large

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doses of insulin to severe diabetic patients. Assuming a constancy in the amounts of protein in the serum, the studies of Peters and his associates (4) (5) indicate that during diabetic ketosis the deficit in chloride and total base is greater than the diminished concentrations of these components would indicate.

The present study was designed to determine, by direct methods, the changes in content of components in the serum of dogs during various stages of experimental diabetes mellitus induced by pancreatectomy.

PROCEDURE. Large, healthy dogs were acclimatized in their cages for a week or more before studies were made. After this preliminary period and a fasting period of 24 hours, the animals were given nembutal parenterally, following which measurement of serum volume was made and blood was obtained for chemical analyses. Total pancreatectomy was then performed. After operation the animals were given maintenance dosages of insulin until recovery from the operation had occurred.

After the animals were in good condition, insulin therapy was completely withdrawn and the animals were permitted to go into ketosis. Final measurements were made when ketonuria was well developed but before circulation became impaired. The period of time required for the development of severe ketosis varied greatly with individual dogs, some remaining active for as long as 3 weeks following withdrawal of insulin. All of the animals had heavy ketonuria for several days before the final measurements were made, although they did not all yield greatly diminished concentrations of CO_2 in the serum. Reliance could not be placed entirely upon the level of CO_2 concentration in the serum in establishing the condition of an animal. Two animals died before the final observations could be made and in one of these the CO_2 concentration was 35 volumes per cent shortly before death.

Since all of the dogs suffered severe reductions in body weight, the effect of simple starvation was studied in 2 animals. Measurements of serum volume and serum electrolytes were made in these dogs before and after losing approximately 25 per cent of their body weights.

The measurement of serum volume was made with vital red using the Sunderman and Austin method (6). Measurement of total base was made by means of the procedure recommended by Hald (7). Estimation of the extracellular water was made by injection of NaCNS following the method of Crandell and Anderson (8). Fatty acids were analyzed by the Mann and Gildea (9) method. Methods for the other analyses have been given in previous publications from this laboratory (10).

In 2 animals portions of rectus muscle (2 to 3 grams net weight) were removed before pancreatectomy and before ketosis. The amounts of solids in these sections were obtained by drying them to constant weight at 105°C.

RESULTS. The results of the serum analyses are given in table 1. It will be observed that from the time of the initial measurements, when the dogs were in a normal healthy state, until the final measurement there was a loss of body weight amounting to from 25 to 43 per cent.

The initial measurements of serum volume expressed in relation to body weight were all practically within the same range of values obtained previously on other normal dogs in this laboratory (45 to 55 ml. per kgm. of body weight). During ketosis the total amounts of serum were decreased in all of the dogs. The serum volumes in relation to body weights were however all increased. Although no initial measurement was obtained in dog 2, nevertheless the determination made during ketosis in this animal yielded a serum volume in relation to body weight that was considerably above the upper normal level. The serum volumes per kilogram of body weight following starvation in dogs 7 and 8 were practically the same as initially,—the total amounts of serum obviously being diminished.

The concentrations of serum *sugar* were increased in all of the depancreatized animals during ketosis and the CO_2 concentrations decreased. The concentrations of *urea nitrogen* were measured in 4 animals and found to be increased during ketosis in 3 of them. In all of the dogs excepting dog 6 the concentrations of serum *solids* and *protein* were decreased in ketosis,—the water per kilo of serum being increased. In table 1 are given the amounts of water per kilo of serum determined from specific gravity data using the conversion formula of Sunderman (11). Although the original factors were obtained from measurements on human sera it will be seen that when applied to dog sera the calculations derived from the formula agree satisfactorily with the values obtained by analysis.

The concentrations of serum *fatty acids* were increased during ketosis in 3 animals in which they were measured. The concentrations of *total base*, *chloride*, and CO_2 were decreased in the sera of all of the depancreatized dogs during ketosis. In the sera of 3 of the experimental animals, measurements of the freezing point were made. During ketosis the *freezing point depressions* of the sera were increased above those obtained initially. The decrease in osmolar concentrations of total base during ketosis was more than compensated by the increased osmolar concentrations of serum sugar and other non-electrolytes.

The concentration of undetermined anions was obtained by adding together the values of BCl, $BHCO_3$ and BPr[2] and subtracting this sum from the total base value. In all of the dogs excepting dog 2 the concentrations of undertermined anions were increased during ketosis.

Since all of the diabetic dogs suffered severe reductions in body weight

[2] $BHCO_3 = [CO_2] - 1.27$
BPr $= 0.97(Pr)(pH - 5.26)$

TABLE 1

Concentration of serum components

DOG	DATE		BODY WEIGHT	SERUM VOLUME		SUGAR	UREA N	SOLIDS	PROTEIN	SP. GR.	WATER		FATTY ACIDS	CHOLESTEROL	TOTAL BASE	CHLORIDE	CO_2	FREEZING POINT	UNDETERMINED ANION
											By analysis	From sp. gr.							
			kgm.	*ml.*	*ml./ kgm. B.W.*	*mg./ 100 ml.*	*mg./ 100 ml.*	*gm/100 gm.*	*gm./ 100 ml.*	*20°/20°*	*gm./ kgm.*	*gm./ kgm.*	*mM/ l.*	*mg./ 100 ml.*	*m.eq./ l.*	*m.eq./ l.*	*vol. per cent*	*°C.*	*m.eq./ l.*
1	9/28	Control before pancreatectomy	11.1	628	56.9	88	24	7.99	6.0	1.0245	920	921	7.8	184	149.6	107.5	58	−0.567	5.9
	10/20	Ketosis after pancreatectomy	7.5	606	80.6	440	30	6.21	4.8	1.0210	938	932	12.4	167	140.0	99.8	49	−0.597	10.3
2	10/22	Control before pancreatectomy	16.2			117	27	7.23	5.2	1.0223	928	927	9.7	258	145.2	107.6	57	−0.565	3.3
	11/21	Ketosis after pancreatectomy	9.2	737	80.1	520	18	6.27	4.8	1.0209	937	932	20.3	208	135.0	105.8	41	−0.581	2.9
3	1/19	Control before pancreatectomy	18.4	974	52.9	110	19	7.21	5.2	1.0222	928	927	10.3	200	146.1	109.8	55	−0.562	3.0
	2/15	Ketosis after pancreatectomy	12.7	873	68.6	430	42	6.43	4.8	1.0210	936	932	18.2	200	144.4	100.3	34	−0.600	20.9
4	6/23	Control before pancreatectomy	19.7	918	46.6	116	20		4.9						149.1	111.2	58		3.8
	8/6	Ketosis after pancreatectomy	12.6	669	53.3	460	29		4.5						137.9	99.4	31		17.2
5	11/29	Control before pancreatectomy	15.8	873	55.3	104		6.79	5.0	1.0217	932	930		125	149.5	112.4	59		2.4
	12/16	Ketosis after pancreatectomy	11.3	818	72.7	440		6.40	4.5	1.0201	936	936		176	136.6	98.5	39		13.3
6	12/17	Control before pancreatectomy	33.8	1780	52.5	100		7.81	5.9	1.0243	922	922		143	147.1	107.6	56		4.2
	1/4	Ketosis after pancreatectomy	25.5	1696	66.7	412		8.36	6.7	1.0265	916	914		169	144.6	105.2	27		15.6
Average values		Controls before pancreatectomy			52.8	106	22	7.44	5.4	1.0230	926	925	9.3	182	147.8	109.4	57	−0.565	3.8
		Ketoses after pancreatectomy			70.3	450	30	6.72	5.0	1.0219	933	929	17.0	184	139.8	101.5	37	−0.593	13.4
7	3/30	Before starvation	16.2	864	53.3	111		8.02	6.3	1.0253	920	918		183	151.6	110.3	55		5.9
	4/18	After starvation	12.1	655	54.4	102		7.42	5.8	1.0239	926	923		151	149.2	106.6	47		11.6
8	1/4	Before starvation	18.6	956	51.4	127		8.20	6.3	1.0253	918	918		104	152.0	106.5	64		6.2
	1/29	After starvation	13.6	654	48.1	119		7.58	5.8	1.0238	924	924		131	156.5	113.3	58		7.5
Average values		Before starvation			52.4	119		8.1	6.3	1.0253	918	918		144	151.9	108.4	60		6.1
		After starvation			51.3	111		7.5	5.8	1.0239	925	924		141	152.9	110.0	53		9.6

comparisons of similar measurements were made in dogs subjected to simple starvation. The results of these measurements are given in table 1. With the exception of reduction in concentrations of serum protein the values for other serum components exhibited no essential change during starvation.

The amounts of components in the circulating serum were calculated during the control period and during ketosis and have been compared in two ways. First, the total amount of a given component in the serum during ketosis was compared to the total amount of that component present during the control measurements. Second, the total amount of a given component per kilogram of body weight during ketosis was compared to the total amount of that component per kilogram of body weight during the control measurements. In table 2 is given the average of the percentile

TABLE 2

Average of percentile changes in serum contents during ketosis

	WEIGHT	SERUM VOLUME	WATER	TOTAL BASE	Cl	BPr	$BHCO_3$	SUGAR	CHOLESTEROL
Diabetes									
Total change	−33	−10	−0.8	−15	−16	−16	−45	+282	+6
Change per unit of body weight		+29	+33	+23	+25	+20	−17	+431	+49
Starvation									
Total change	−26	−28	−31	−30	−28	−20	−38	−31	−14
Change per unit of body weight		−2	−2	−2	−1	−13	−7	−7	−16

changes in the serum contents during ketosis as related *a*, to the total amount present in the circulation during the control period, and *b*, as related to the amount present per kilogram of body weight during the control period. The values in the depancreatized animals are compared with those obtained in the starved animals.

From the time of the initial to the final measurements the diabetic dogs lost weight amounting to from 25 to 43 per cent with an average loss of 33 per cent. Final measurements were made in the starved animals after loss of 26 per cent of the body weight.

It will be seen that although the actual amount of serum present in the circulation during diabetic ketosis was 10 per cent less than that in the healthy state, the serum volume per kilogram of body weight was on the average 29 per cent greater than that in the healthy state. In the starved animals the total serum volume was decreased in proportion to the reduction in weight and within the limits of error of our measurements the serum volume per kilogram of body weight was unchanged.

In the diabetic dogs the total amount of water in the serum during ketosis was unchanged although the serum water per unit of body weight was increased 33 per cent. In the starved dogs the total serum water was decreased in proportion to the decrease in body weight, so that the serum water per unit of body weight was essentially unchanged. The actual amounts of total base, Cl, and BPr were all decreased during ketosis, although the amounts of these components per unit of body weight were all increased. In the starved animals the total amounts of these components, excepting BPr, exhibited essentially no change during ketosis when expressed in relation to the body weight.

TABLE 3

Extracellular water in dogs

DOG		BODY WEIGHT	EXTRACELLULAR WATER		SOLIDS RECTUS MUSCLE
		kgm.	*kgm.*	*per cent of body weight*	*gm./100 grams wet tissue*
5	Control	15.3	4.89	31.0	24.8
	Diabetic ketosis	11.3	4.89	43.5	23.8
6	Control	33.8	10.64	31.5	26.6
	Diabetic ketosis	25.5	10.16	39.9	26.1
7	Control	16.2	5.44	33.6	
	Starvation	12.1	4.51	37.3	
9	Control	28.4	8.59	30.2	
10	Control	27.1	7.87	29.1	
11	Control	21.6	6.26	29.0	

From the data in table 2 it will be seen that of all the serum components studied in the diabetic dog and expressed in relation to body weight, the bicarbonate content alone per kilogram of body weight was consistently decreased. These analyses of the serum in the depancreatized animals are in marked contrast to those obtained during the ketosis of starvation. In the starved animals the amounts of the serum components as well as the serum volume per unit of body weight remained practically unchanged with the exception of protein and cholesterol which were significantly decreased.

Extracellular water. The results of the measurements of extracellular water in dogs 5, 6 and 7 are given in table 3. The concentrations of thiocyanate were calculated in relation to the serum water employing the conversion factors obtained from specific gravity data (11). The amount

of extracellular water was calculated in each case by dividing the amount of NaCNS injected by the concentration of thiocyanate in the serum water, making allowance for any thiocyanate excreted in the urine during the one hour interval between the times of administration of NaCNS and the collection of the blood sample. In addition to the 3 estimations obtained during the control periods in dogs 5, 6 and 7, three measurements of extracellular water were made in normal dogs 9, 10 and 11 and these results are included in table 3. The average of 6 estimations of the extracellular water in normal dogs was 30.7 ± 1.4 per cent of the body weight.

During ketosis in the depancreatized dogs 5 and 6 the percentage of extracellular water was increased by 12.5 and 8.4 per cent respectively. It would seem noteworthy that in both of these animals the actual quantity of extracellular water present during this period was practically the same as during the control period. In dog 7 the percentage of extracellular water was increased 3.7 per cent during starvation.

From the data in table 3 it will also be observed that the percentages of solids contained in rectus muscle during ketosis in dogs 5 and 6 were only slightly less than those obtained during the control period,—the percentages of muscle water thus being only slightly increased during ketosis.

Discussion. It would seem noteworthy that our findings during ketosis in depancreatized dogs of increased serum water and extracellular water per kilo of body weight are the opposite to similar measurements obtained in adrenal cortical insufficiency. In the latter condition the serum volume (12) and extracellular fluids are diminished (13) and the studies of Harrop and others suggest that the percentage of intracellular water is increased. Swingle and his associates (14) concluded that the intracellular fluids are increased in experimental adrenal insufficiency and that following the administration of cortin to adrenalectomized dogs the accumulation of intracellular fluid and electrolytes shifts from the intracellular to the extracellular and vascular compartments.

The question may be raised as to whether our observations during ketosis in depancreatized dogs are similar to those obtained during severe ketosis in diabetes mellitus. Certainly the changes in concentrations of the serum components are analogous to those obtained in humans. It is difficult to evaluate degrees of dehydration in dogs; however, these animals presented dry mucous membranes and desired to ingest large amounts of water. It should be noted that they had free access to water at all times.

Our data do not permit calculation of the water in the intracellular fluid compartment. However, since the animals at the time of our measurements during ketosis appeared dehydrated an inference might be drawn that, if the body water per kilogram of body weight were diminished, water must have been lost from the intracellular fluid compartment.

Chang, Harrop and Schaub's observations of decreased circulating

blood and plasma volumes during ketosis in diabetes mellitus may not necessarily be inconsistent with our observations. These workers reported diminution in the total blood and plasma volumes in 5 individuals with severe acidosis when compared to the values obtained during recovery. It would seem possible that the values for blood volume obtained during recovery in their patients might not necessarily represent the values that might have been obtained after they were controlled for a longer period or at a time when they had not been suffering from diabetes mellitus.

SUMMARY

Measurements of the serum volume, extracellular fluid and various serum components were made in totally depancreatized dogs that had been permitted to go into ketosis following the withdrawal of insulin.

The serum volume per kilogram of body weight in our animals was found to be consistently increased during ketosis. The total volumes of serum in the circulation were, however, decreased when compared to the total volumes measured during the healthy state. Although the concentrations of electrolytes were consistently diminished during ketosis, nevertheless, owing to the increase in serum volume per kilo of body weight, the total amounts of the serum electrolytes (excepting $BHCO_3$) per kilogram of body weight were found to be actually increased. Our studies also indicated that during diabetic ketosis the extracellular water per unit of body weight was increased.

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THE EFFECT OF HYPOXEMIA ON VENTILATION AND CIRCULATION IN MAN

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The study of the factors controlling respiration and circulation has made much progress in the last two decades. Hering (1927), Koch (1933) and Heymans (1933) have opened new ways of investigation and made possible a more detailed study of the effect of carbon dioxide, oxygen and hydrogen-ion on both ventilation and circulation. Grollman's introduction of acetylene in cardiac output determinations made the original Krogh-Lindhard method easier to use and Christensen (1937) has shown that it remains the most reliable and handy method for use on human subjects.

All the reviews of the control of *respiration* (Nielsen (1936), Heymans and Bouckaert (1939), Gesell (1939) and Schmidt and Comroe (1940)) emphasize the dominant rôle of the tensions of the CO_2, of the O_2 and the H-ion concentration. It has not yet been possible to isolate as satisfactorily factors which control the *circulation* but evidently the same three also play very important rôles (Grollman (1932), Christensen (1937), McDowall (1938), Heymans and Bouckaert (1939) and others). It is the purpose of this paper to describe the effects of oxygen deficiency on the respiration and the circulation of normal men in rest and during work.

Oxygen deficiency has been evoked in two radically different ways, one by replacing part of the HbO_2 with HbCO, the other by letting the subject breathe a gas mixture low in oxygen. The data will be interpreted and discussed in the light of present hypotheses regarding the regulation of ventilation and circulation. For the sake of clearness, respiration and circulation will be treated separately.

A. Respiration. Oxygen-lack evokes hyperventilation as pointed out by Haldane and Priestley (1905). This has been confirmed many times since then, and the mechanism of its effect on the ventilation has been carefully studied by, for instance, Lindhard (1911), Barcroft (1925), Nielsen (1936), Gesell (1939), and many others. The introduction of Heymans' technique again stimulated research in this field: numerous reports have been published on the effect of O_2-lack on the carotid glomus (see Heymans and Bouckaert (1939) and Schmidt and Comroe (1940)).

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Procedure and methods. For all experiments the subjects came fasting to the laboratory in the morning. In rest the subjects were lying on a bed, and no determinations were made during the first hour. We thus made sure that we got basal and easily reproducible conditions. In work (walking on a motor-driven treadmill) no determinations were made before a steady state was reached. The subject breathed through mouthpiece and valve, the expired air was collected and measured in a Tissot-gasometer and a sample of it analyzed on the Haldane apparatus. The total ventilation was reduced to 0°, 760 mm. Hg and dryness for calculating the metabolism, and to 37°, prevailing barometric pressure and complete saturation for estimating the ventilation per minute. Alveolar air samples were taken in rest by the Haldane-Priestley method, during work by means of the modified Lindhard procedure.

The CO was prepared from HCOOH and H_2SO_4 and collected over water. For administration a measured amount (usually between 350 and 450 cc.) was introduced into a Roth-Benedict apparatus, which afterwards was filled with oxygen. The subject was connected with the apparatus in the ordinary way and rebreathed the mixture for 30 to 45 minutes. A refilling with oxygen was necessary after about 20 minutes. About 10 minutes after disconnecting the subject from the Roth-Benedict apparatus a sample of venous blood was drawn and the experiment on the CO-poisoned subject began. Immediately after the end of the experiment a second sample of blood was taken. The CO contents were determined on the Van Slyke apparatus after Van Slyke and Neill (see Peters and Van Slyke, p. 328, 1932). The CO-content of the blood during an experiment is given as the average of the two determinations made just before and just after the experiment. The oxygen capacity of the blood was estimated from the cell volume by means of the formula of Dill, Edwards and Consolazio (1937). Blood samples (venous or capillary) were drawn for blood-lactate determinations after Edwards (1938). In some cases direct pH measurements on arterialized capillary blood were made by means of the glass electrode by Dr. W. H. Forbes.

Most of the experiments were carried out on three subjects, E. A., 32 years, 171 cm., 69 kgm.; H. C., 32 years, 170 cm., 75 kgm.; and B. C., colored, 20 years, 170 cm., 64 kgm. Normal oxygen dissociation curves had previously been determined for each subject.

Results. The results of our experiments on the three subjects, E. A., H. C. and B. C., are averaged in table 1. Figure 1 gives a graphic representation of the average ventilations plotted against the oxygen intake. The individual determinations were so close to each other that a separate presentation of them in a figure was impossible.

In table 1 the ventilations are given at 37°, observed barometric pressure and saturation. It is evident that the ventilations in rest and during

TABLE 1

SUBJECT	STATE	REST							LIGHT WORK							HEAVY WORK						
		Ventilation		Alveolar Air		Arterial O_2		Blood lactate	Ventilation		Alveolar Air		Arterial O_2		Blood lactate	Ventilation		Alveolar air		Arterial O_2		Blood lactate
				pCO_2	pO_2						pCO_2	pO_2						pCO_2	pO_2			
		l./min.	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*	*l./min.*	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*	*l./min.*	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*
E. A.	Normal	4.7	19.7	42	96	17.9	96	12	21.6	20.8	41	100	17.9	96	10	38.6	21.3	45	99	17.9	96	13
	CO-poisoned	4.8	19.1	41	99	12.1	65	13	21.6	19.8	41	99	12.7	68	13	39.4	22.4			13.0	70	24
	Breathing low O_2 mixture	8.0	35.6	34	36	12.6	68		33.3	32.3	29	40	13.8	74	14	61.0	38.1	30	45	14.9	80	53
H. C.	Normal	6.0	27.1	43	101	18.2	96	12	26.9	22.8	37	106	18.2	96	10	41.9	22.7	44	98	18.2	96	21
	CO-poisoned	6.5	29.4	43	97	12.6	66	9	25.0	22.1	41	103	13.0	68	16	62.0	31.0	32	117	13.3	70	81
	Breathing low O_2 mixture	8.8	37.6	33	38	13.3	70		35.8	34.8	28	46	15.4	81	28	84.4	52.8	25	52	16.5	87	107
B. C.	Normal	6.4	27.2	40	102	19.2	96	12	26.3	26.8	34	107	19.2	96	16	35.6	23.7			19.2	96	15
	CO-poisoned	6.0	24.8	41	106	12.7	63	12	25.0	25.8	35	105	14.6	73	14	35.7	25.3			14.0	70	36
	Breathing low O_2 mixture	9.8	43.5	30	38	13.0	65															

TABLE 2

SUBJECT	STATE	REST					LIGHT WORK					HEAVY WORK				
		O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.	O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.	O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.
		l.	*cc./l.*	*l./min.*		*cc.*	*l.*	*cc./l.*	*l./min.*		*cc.*	*l.*	*cc./l.*	*l./min.*		*cc.*
E. A.	Normal	0.239	47	5.1	59	86	1.04	92	11.3	99	114	1.81	109	16.6	120	138
	CO-poisoned	0.247	44	5.6	64	87	1.09	81	13.5	128	105	1.76	102	17.3	159	109
	Breathing low O_2 mixture	0.225	24	9.4	73	129	1.03	47	21.9	132	166	1.60	54	29.6	159	186
H. C.	Normal	0.221	52	4.3	58	74	1.18	103	11.5	105	110	1.85	128	14.5	150	97
	CO-poisoned	0.221	48	4.6	71	65	1.13	94	12.0	146	82	2.00	129	15.5	186	83
	Breathing low O_2 mixture	0.234	29	8.1	76	107	1.03	67	15.4	149	103	1.60	86*	18.6	184	101
B. C.	Normal	0.235	58	4.1	57	72	0.98	101	9.7	91	107	1.50	121	12.4	134	93
	CO-poisoned	0.242	52	4.7	68	69	0.97	102	9.5	120	79	1.41	119	11.9	174	68
	Breathing low O_2 mixture	0.225	23	9.8	71	138										

* Only two determinations, 85 and 87, both too high; cardiac output and stroke volume accordingly too low.

light work in all three subjects are virtually unaffected by CO hypoxemia. In the heavy work the same is the case in E. A. and B. C., whereas H. C. shows a rather large increase. The last column shows that this subject alone had a significant increase in blood lactate during this type of work (to 81 mgm. per cent). In low oxygen without CO-poisoning all three subjects showed marked increases in ventilation in rest. Both E. A. and H. C. could perform the work long enough to reach a steady state, also in low oxygen, and both show important increases in ventilation and—in the heavy work—in blood lactate. The third subject, B. C., could not continue work in low oxygen long enough to get any reliable determinations of his ventilation.

The alveolar CO_2 and O_2 pressures are normal in the CO experiments, except in the case of H. C. during heavy work, where the blood lactate was increased. In low oxygen (about 10 per cent O_2 in the inspired air) the pCO_2 dropped from around 40 mm. Hg to around 30 mm. Hg.

The arterial oxygen content in the control experiments is calculated on the assumption that the arterial blood is 96 per cent saturated with oxygen. In the CO experiments the "available Hb" is found by subtracting the amount bound to CO, as found by the Van Slyke analyses, from the total Hb. Of the "available Hb" 96 per cent is assumed to be in the oxygenated form. For the low O_2 experiments the oxygen saturation is derived from the individual oxygen dissociation curves of the subjects, using the alveolar pO_2 as the O_2 tension of the arterial blood. It will be seen that the oxygen content of the arterial blood was decreased to approximately the same degree in the resting experiments under CO and with low O_2; in the work experiments the oxygen content usually was less decreased in low O_2 than in the CO experiments.

Figure 1 shows that the increase in ventilation with increasing oxygen consumption takes place in practically the same way in normal subjects and in subjects where 20 to 30 per cent of the Hb is saturated with CO, whereas the ventilation in low O_2 starts at a higher level and increases faster than in the two other series.

Discussion. Our results show that there is a markedly different respiratory response to the two forms of hypoxemia investigated, despite the fact that in each case 20 to 30 per cent of the hemoglobin is unsaturated with O_2. It is reasonable to assume that in all tissues where oxygen is used—e.g., in the muscles, the heart, and in the brain—about the same initial degree of oxygen deficiency must have occurred. A hypoxemia can, of course, be counteracted by an increase of the blood flow, but—as will be shown later—the blood flow is not accelerated in the CO experiments, whereas in low O_2 it is considerably increased. The oxygen deficiency of the tissues—including the brain—consequently should be more severe in the anemic hypoxemia (CO-experiments) than in the hypoxic

| Breathing low O_2 mixture | 0.225 | 23 | 9.8 | 71 | 138 |

* Only two determinations, 85 and 87, both too high; cardiac output and stroke volume accordingly too low.

TABLE 1

SUBJECT	STATE	REST							LIGHT WORK							HEAVY WORK						
		Ventilation		Alveolar Air		Arterial O_2		Blood lactate	Ventilation		Alveolar Air		Arterial O_2		Blood lactate	Ventilation		Alveolar air		Arterial O_2		Blood lactate
				pCO_2	pO_2						pCO_2	pO_2						pCO_2	pO_2			
		l./min.	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*	*l./min.*	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*	*l./min.*	*l./l.O₂*	*mm. Hg*	*mm. Hg*	*vol. per cent*	*per cent sat.*	*mgm. per cent*
E. A.	Normal	4.7	19.7	42	96	17.9	96	12	21.6	20.8	41	100	17.9	96	10	38.6	21.3	45	99	17.9	96	13
	CO-poisoned	4.8	19.1	41	99	12.1	65	13	21.6	19.8	41	99	12.7	68	13	39.4	22.4			13.0	70	24
	Breathing low O_2 mixture	8.0	35.6	34	36	12.6	68		33.3	32.3	29	40	13.8	74	14	61.0	38.1	30	45	14.9	80	53
H. C.	Normal	6.0	27.1	43	101	18.2	96	12	26.9	22.8	37	106	18.2	96	10	41.9	22.7	44	98	18.2	96	21
	CO-poisoned	6.5	29.4	43	97	12.6	66	9	25.0	22.1	41	103	13.0	68	16	62.0	31.0	32	117	13.3	70	81
	Breathing low O_2 mixture	8.8	37.6	33	38	13.3	70		35.8	34.8	28	46	15.4	81	28	84.4	52.8	25	52	16.5	87	107
B. C.	Normal	6.4	27.2	40	102	19.2	96	12	26.3	26.8	34	107	19.2	96	16	35.6	23.7			19.2	96	15
	CO-poisoned	6.0	24.8	41	106	12.7	63	12	25.0	25.8	35	105	14.6	73	14	35.7	25.3			14.0	70	36
	Breathing low O_2 mixture	9.8	43.5	30	38	13.0	65															

TABLE 2

SUBJECT	STATE	REST					LIGHT WORK					HEAVY WORK				
		O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.	O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.	O_2/min.	A-v. O_2-diff.	Cardiac output	Pulse rate	Stroke vol.
		l.	*cc./l.*	*l./min.*		*cc.*	*l.*	*cc./l.*	*l./min.*		*cc.*	*l.*	*cc./l.*	*l./min.*		*cc.*
E. A.	Normal	0.239	47	5.1	59	86	1.04	92	11.3	99	114	1.81	109	16.6	120	138
	CO-poisoned	0.247	44	5.6	64	87	1.09	81	13.5	128	105	1.76	102	17.3	159	109
	Breathing low O_2 mixture	0.225	24	9.4	73	129	1.03	47	21.9	132	166	1.60	54	29.6	159	186
H. C.	Normal	0.221	52	4.3	58	74	1.18	103	11.5	105	110	1.85	128	14.5	150	97
	CO-poisoned	0.221	48	4.6	71	65	1.13	94	12.0	146	82	2.00	129	15.5	186	83
	Breathing low O_2 mixture	0.234	29	8.1	76	107	1.03	67	15.4	149	103	1.60	86*	18.6	184	101
B. C.	Normal	0.235	58	4.1	57	72	0.98	101	9.7	91	107	1.50	121	12.4	134	93
	CO-poisoned	0.242	52	4.7	68	69	0.97	102	9.5	120	79	1.41	119	11.9	174	68
	Breathing low O_2 mixture	0.225	23	9.8	71	138										

* Only two determinations, 85 and 87, both too high; cardiac output and stroke volume accordingly too low.

light work in all three subjects are virtually unaffected by CO hypoxemia. In the heavy work the same is the case in E. A. and B. C., whereas H. C. shows a rather large increase. The last column shows that this subject alone had a significant increase in blood lactate during this type of work (to 81 mgm. per cent). In low oxygen without CO-poisoning all three subjects showed marked increases in ventilation in rest. Both E. A. and H. C. could perform the work long enough to reach a steady state, also in low oxygen, and both show important increases in ventilation and—in the heavy work—in blood lactate. The third subject, B. C., could not continue work in low oxygen long enough to get any reliable determinations of his ventilation.

The alveolar CO_2 and O_2 pressures are normal in the CO experiments, except in the case of H. C. during heavy work, where the blood lactate was increased. In low oxygen (about 10 per cent O_2 in the inspired air) the pCO_2 dropped from around 40 mm. Hg to around 30 mm. Hg.

The arterial oxygen content in the control experiments is calculated on the assumption that the arterial blood is 96 per cent saturated with oxygen. In the CO experiments the "available Hb" is found by subtracting the amount bound to CO, as found by the Van Slyke analyses, from the total Hb. Of the "available Hb" 96 per cent is assumed to be in the oxygenated form. For the low O_2 experiments the oxygen saturation is derived from the individual oxygen dissociation curves of the subjects, using the alveolar pO_2 as the O_2 tension of the arterial blood. It will be seen that the oxygen content of the arterial blood was decreased to approximately the same degree in the resting experiments under CO and with low O_2; in the work experiments the oxygen content usually was less decreased in low O_2 than in the CO experiments.

Figure 1 shows that the increase in ventilation with increasing oxygen consumption takes place in practically the same way in normal subjects and in subjects where 20 to 30 per cent of the Hb is saturated with CO, whereas the ventilation in low O_2 starts at a higher level and increases faster than in the two other series.

Discussion. Our results show that there is a markedly different respiratory response to the two forms of hypoxemia investigated, despite the fact that in each case 20 to 30 per cent of the hemoglobin is unsaturated with O_2. It is reasonable to assume that in all tissues where oxygen is used—e.g., in the muscles, the heart, and in the brain—about the same initial degree of oxygen deficiency must have occurred. A hypoxemia can, of course, be counteracted by an increase of the blood flow, but—as will be shown later—the blood flow is not accelerated in the CO experiments, whereas in low O_2 it is considerably increased. The oxygen deficiency of the tissues—including the brain—consequently should be more severe in the anemic hypoxemia (CO-experiments) than in the hypoxic

* Only two determinations, 85 and 87, both too high; cardiac output and stroke volume accordingly too low.

hypoxemia (low O_2-experiments), and nevertheless it is in the last case we find an increase in the ventilation. General oxygen-lack in the tissues—muscles, brain, etc.—therefore cannot be assumed to cause the hyperventilation.

This result is in good agreement with the experiments upon animals, where it often has been demonstrated that perfusion of the brain with hypoxic blood does not produce any hyperventilation, but, on the contrary, frequently a hypoventilation (Heymans and Bouckaert, Schmidt and Comroe). Quite different is the result when the *carotid body* is perfused with hypoxic blood: in that case a marked hyperventilation is always found, and the importance of the carotid body as an accessory to the respiratory center is now a well-established fact. It is therefore quite

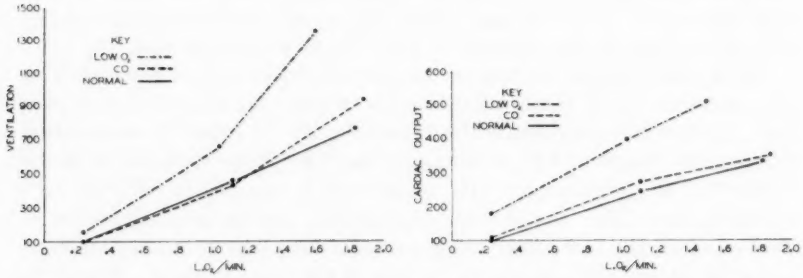

Fig. 1. Left. The ventilations of E. A. and H. C. averaged and plotted against the O_2-consumption. The ventilations are shown as percentages of the normal, resting value.

Fig. 2. Right. The cardiac outputs of E. A. and H. C. averaged and plotted against the O_2-consumption. The cardiac outputs are shown as percentages of the normal, resting value.

natural to assume that the effect of the low oxygen also in man is evoked through the chemoreceptors of the carotid body.

The most common explanation of the way in which hypoxemia affects the chemoreceptors is to assume that it produces a local oxygen-lack in the cells of the glomus. This explanation can be maintained if we assume that the oxygen consumption of the glomus is low in relation to the arterial supply, which we know is abundant, so that the reductions in combined O_2 and hence in pO_2 are small. This means that in CO-poisoning the pO_2 within the glomus is not much below its normal values. When the pO_2 of arterial blood supplied the glomus is low—as when a low O_2 mixture is breathed—the pO_2 within the glomus will be even lower, and there is a strong stimulus transmitted to the respiratory center.

Table 1 shows that in work experiments where the blood lactate was increased beyond about 50 mgm. per cent the ventilation was increased

out of proportion to the increase in metabolic rate. However, it is not possible to draw any conclusions from our data as to the effect of lactate accumulation on the ventilation except this simple statement. It might, though, be of interest to note that in the very few cases in which direct pH determinations on arterialized capillary blood were made (during work in low O_2) the pH was found to be normal or increased, even when the blood lactate was as high as 53 mgm. per cent. The acid effect of lactate accumulation must have been balanced or out-weighed by the buffer action of proteins, and the elimination of extra carbonic acid by hyperventilation.

B. Circulation. The effect of hypoxemia on the circulation has been widely studied. Most observations, however, have been made on the pulse rate and on blood pressure, rather few on the circulation rate. Hasselbalch and Lindhard (1915) made some determinations of the cardiac output in a low pressure chamber by means of the nitrous-oxide method of Krogh and Lindhard but found no increase in the circulation rate, presumably because the O_2 tension was not low enough. Douglas, Haldane, Henderson and Schneider (1913) and Barcroft and co-workers (1923) found no effect of high altitude on the cardiac output, but their methods were inferior to those used later. Ewig and Hinsberg (1930) and Grollman (1930) found an increase of the cardiac output in the first days of a sojourn in high altitude, as did Christensen and Nielsen (1936) and Christensen and Forbes (1937) both in a low pressure chamber and in high altitude. The general result of the present observations in low O_2 seems to be the one set forth by Christensen and Forbes: There is an initial increase of the cardiac output when the O_2 tension is low enough, but prolonged (i.e., lasting several days) exposure is followed by a secondary return of the cardiac output to normal sea level values.

A few observations on anemic hypoxemia (e.g., H. E. Nielsen (1934) on man) indicate that prolonged anemia at least is accompanied by an increased cardiac output.

As there seems to be a difference between the effects of acute and more prolonged hypoxemias on the cardiac output, it might be worth mentioning that the present paper deals only with the acute forms of hypoxemia.

Procedure and methods. The subjects were the same as in the previously described experiments, and so were the precautions taken to secure reproducible conditions. Pulse rates were obtained in rest by counting the pulse at the wrist for half a minute, in work by counting the clicks of a cardiotachometer, which picked up and amplified the action potential of the heart. Blood pressures were measured in rest at the left upper arm by the ordinary auscultatory method. The cardiac output was estimated from the rate of the O_2 consumption and the arteriovenous O_2-difference, which was obtained by means of Christensen's modification of

Grollman's acetylene method. When used with the prescribed precautions (see Christensen (1937) for details) this method gives reliable results in rest and in work, when the alveolar O_2 tension is normal or above normal. When the alveolar O_2 tension is lower than normal, special precautions must be taken to make sure that the average O_2 tension in the gas samples taken during the determination is about the same as the alveolar O_2 tension observed beforehand (Lindhard, 1915; Grollman, 1930; Christensen and Nielsen, 1936). Grollman (1930) suggests a correction for deviations from this rule by means of the oxygen dissociation curve or by interpolation, and Christensen and Nielsen (1936) think that a determination of the oxygen intake during the rebreathing period will give a control of the reliability of the determination. In our opinion, neither of these methods of correction is reliable. We therefore prefer, in the experiments with low O_2, to use only those values of the a-v O_2 difference during the determination of which the average O_2 tension was equal to, or very close to, the alveolar O_2 tension. The procedure in the low O_2 experiments, therefore, was first to secure a sample of alveolar air, as described before; then, after some minutes, to let the subject exhale deeply, have him quickly change over to the mouthpiece of the Grollman bag, and then to perform the experiment in the usual way. In the experiments with partial saturation of the Hb with CO, no such precautions are necessary, as the available Hb in this case will be completely saturated with O_2 in passing the lungs.

Results. Table 2 and figure 2 show the average data of our experiments. It will be seen that the a-v O_2 differences in normal conditions, and with about 30 per cent HbCO in the blood, both in rest and during work are very similar, whereas the a-v O_2 difference in low O_2 is greatly diminished. Accordingly, the cardiac output with CO is only slightly increased, whereas in low O_2 it is significantly increased. The pulse rates do not correspond to this difference: high pulse rates are found both with CO and in low O_2, which demonstrates the well-known fact that the pulse rate cannot always be taken as an indicator of the rate of the blood flow. The stroke volume shows a very interesting fact: it is lower than normal with CO, equal to or higher than normal in low O_2. The blood pressure, measured only in rest, was approximately normal in low O_2 and showed a slight tendency to an increase with CO.

It is necessary, during the discussion, to mention the average capillary O_2 tension. A direct measurement of this has not been made, but it can be assumed to have been close to the average venous O_2 tension. An estimation of this is shown for one of the subjects in table 3. In table 3 the arterial O_2 content in volumes per cent is taken from table 1 and the arterial O_2 tension is assumed to equal the alveolar O_2 tension in the same table. The venous O_2 content is determined by subtracting the a-v

O_2 difference of table 2, expressed in volumes per cent, from the arterial O_2 content, and the venous O_2 tension is estimated from O_2 dissociation curves of the arterial blood, normal and with 30 per cent of CO saturation (formula in Peters and Van Slyke, p. 618). This introduces an error, as dissociation curves of *venous* blood ought to be used. The difference, however, would even in work be less than 5 mm. Hg, so that for our purpose this approximation will serve. Table 3 shows that whereas the venous O_2 tension in low O_2 is approximately normal, it is very low in the CO experiments.

Discussion. The experiments, the results of which are presented in table 2 and figure 2, show that the two forms of hypoxemia—the anemic in CO-poisoning and the hypoxic in low O_2—interfere with the circulation in different ways.

The only effects of the anemic hypoxemia on the circulation are apparently a high pulse rate and a correspondingly low stroke volume (see

TABLE 3

SUBJECT	STATE	REST				LIGHT WORK				HEAVY WORK			
		Arterial O_2		Venous O_2		Arterial O_2		Venous O_2		Arterial O_2		Venous O_2	
		Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension	Vol.	Tension
		per cent	*mm. Hg*	*per cent*	*mm. Hg*	*per cent*	*mm. Hg*	*per cent*	*mm. Hg*	*per cent*	*mm. Hg*	*per cent*	*mm. Hg*
E. A.	Normal	17.9	96	13.2	37	17.9	100	8.7	28	17.9	99	7.0	25
	CO-poisoned	12.1	99	7.3	16	12.7	99	4.6	11	13.0	100	2.8	7
	Breathing low O_2 mixture	12.6	36	10.2	30	13.8	40	9.1	29	14.9	45	9.5	29

table 2). The cardiac output is practically normal. The increase in pulse rate can be looked upon in at least two different ways: First, it could be assumed to be an unsuccessful attempt by the regulatory mechanisms of the body to increase the circulation. Second, it could be assumed to be part of a regulatory mechanism which keeps the cardiac output from falling below the normal. Against the first assumption speaks the fact that the cardiac outputs in the CO experiments with all three subjects, in rest and during work, are so near normal. This would be a very extraordinary coincidence if the aim of the pulse acceleration were to increase it beyond normal. The second assumption, that the pulse rate is high in order to keep the cardiac output from falling below normal, makes necessary the assertion that the cardiac output would have been subnormal if the pulse acceleration had not taken place. As the cardiac output is the product of pulse rate and stroke volume, this means that the stroke volume of the heart, for one reason or another, is abnormally low in the CO ex-

periments. A subnormal stroke volume is ordinarily the effect of a subnormal venous pressure, which again can be caused by an abnormal distribution of the blood in the vessels. In parallelism to this, it is quite natural to assume that the low stroke volume in the CO hypoxemia is caused by a decreased filling of the central veins, called forth by a pooling of blood in the capillaries, which no doubt at the prevailing low capillary pO_2 (comp. table 3) will be widely dilatated (Krogh, 1929). A low venous pressure would result in lowering the arterial blood pressure, were it not for a pressosensible reflex from the aortic arch and the carotid sinus that compensates for the falling blood pressure by an increased pulse rate. (For a more detailed discussion, see Asmussen, Christensen and Nielsen, 1939, 1940.)

The increased pulse rate in the anemic hypoxemia of CO-poisoning thus can be explained as a pressoregulatory compensation for the vasodilatation. A stimulation of the circulatory center, in an attempt to compensate for the low tissue O_2 tension which caused the vasodilatation, has apparently not taken place. It is therefore reasonable to conclude that acute O_2-lack in the tissues is, neither directly nor indirectly, i.e., via the pressoregulation, a stimulus for the circulatory center.

Quite different is the picture when, as in the hypoxic hypoxemia, the O_2 tension of the arterial blood is low. In this case we find a greatly increased cardiac output, effected by both a pulse acceleration and an increased stroke volume (table 2). The low O_2 content of the arterial blood is compensated for by the increased blood flow, so that the capillary O_2 tension, as indicated by the venous O_2 tension in table 3, remains normal. It is obvious that the low O_2 tension of the arterial blood must have acted as a stimulus on the mechanisms which regulate the circulation.

From experiments on animals it is now well known that low arterial O_2 tension produces pulse acceleration and hypertension if the chemoreceptors of the carotid body are intact, whereas no effect is obtained if the chemoreceptors are denervated (Schmidt and Comroe, 1940). In man presumably the same mechanisms are at hand, so that it is reasonable to assert that the effect of the low arterial O_2 tension on the cardiac output in man is produced via the chemoreceptors of the carotid (and aortic) body.[2]

It is emphasized that this paper has dealt only with *acute* hypoxemias, i.e., hypoxemias lasting only up to a few hours. Prolonged exposures will have quite different effects, as the results from prolonged exposures to low O_2 indicate (Grollman, 1930; Christensen and Forbes, 1937), and as the investigations on patients with pernicious anemia have shown (Nielsen,

[2] The ways in which an increase in cardiac output actually is produced—by emptying the blood depots, pulse acceleration and compensatory vasoconstrictions in idle organs—have been discussed by Krogh (1912) and will not be considered here.

1934). The ways in which this adaptation is effected need further investigation.

SUMMARY

A. Respiration. Our experiments have shown that hypoxemia caused by partial CO-poisoning (20–30 per cent) (anemic hypoxemia) elicits little or no respiratory response whereas hypoxemia caused by an alveolar pO_2 of about 40 mm. Hg (hypoxic hypoxemia) causes a very pronounced hyperventilation both in rest and in work.

It is concluded: 1. General O_2-lack in the tissues does not evoke a hyperventilation. 2. The O_2 *tension* of the arterial blood, and not its O_2 *content*, is the active factor in causing hyperventilation. 3. The effect is produced through the pO_2 of the carotid body (glomus).

B. Circulation. Our experiments have shown that both in rest and during work a state of *hypoxic hypoxemia*, with the O_2 in the inspired air low enough to reduce the arterial HbO_2 to 70 or 80 per cent, produces an increase in cardiac output above the normal. Furthermore, both in rest and during work a state of *anemic hypoxemia* (20–30 per cent Hb saturated with CO) has little or no effect on the cardiac output, but increases the pulse rate considerably.

It is concluded: 1. Acute O_2-lack in the tissues is not a stimulus for the circulation. 2. A lowered O_2 *tension* of the arterial blood, not a lowered O_2 *content*, is a stimulus for the circulation. 3. The effect probably is produced through the chemoreceptors of the carotid glomus.

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PARASYMPATHETIC SENSITIZATION IN THE CAT'S EYE

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In 1921 Loewi (1) discovered that vagal stimulation of a frog's heart liberated a substance which when perfused through another frog's heart induced typical vagal inhibitory effects. This finding was of considerable interest, for it had been noted that acetylcholine mimics the action of the parasympathetic nerve impulses (2). Much experimental evidence suggesting the identity of Loewi's "vagusstoff" and acetylcholine has accumulated (3). The action of this drug can be intensified either by previous treatment of the tissue with physostigmine or by parasympathetic denervation. Under these conditions the cat's pupil constricts strongly when small amounts of acetylcholine are instilled into the conjunctival sac (4), or injected into the anterior chamber of the eye (5).

The following study was designed to investigate the responses of the pupil to acetylcholine after interruption of the pre- and post-ganglionic parasympathetic nerve fibers.

METHODS. All operations were carried out with strict aseptic precautions on cats anesthetized with nembutal (36 mgm. per kgm. body weight, intraperitoneally). The parasympathetically decentralized pupil was obtained by intracranial section of the oculomotor nerve. The nerve was approached by trephining the parietal bone and raising the temporal lobe of the brain from the middle fossa. Denervation of the sphincter was accomplished by excision of the ciliary ganglion (4). No ocular muscle was cut, nor were any of the intraorbital vessels injured. Anderson's observation (6) that certain animals develop a slight opacity of the cornea which gradually disappears was confirmed. The region involved coincided with that part of the cornea which was uncovered when the lids were partly closed. In the later operations corneal injury was avoided by closing the eyes with adhesive tape during the period of anesthesia.

The responses of the circular muscle of the iris to acetylcholine were determined at various intervals after operation. Since the instillation of acetylcholine (1 to 5 per cent) produces an inflammation of the con-

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junctiva, the drug was injected intravenously. The injection of less than 2 mgm. per kgm. of body weight produced slight effects on the sensitized sphincter of the pupil. To obtain constriction consistently, doses of 5 mgm. per kgm. were required. Unless specifically mentioned, no physostigmine was instilled during the sensitization tests. During the acetylcholine tests the cats were not anesthetized.

Changes in pupillary size were usually determined by measuring the horizontal diameter with a millimeter scale. In certain instances the eye was photographed with a synchronized flash light attached to the camera. Lasting but $\frac{1}{715}$ of a second, the flash was well within the latent period of the light reflex (7).

Results. Immediately following intracranial section of the oculomotor nerve (5 cats) or excision of the ciliary ganglion (8 cats), the ipsilateral pupil was markedly dilated. Twenty-four to thirty-six hours after either operation examination of the animals revealed a prominent bulbus despite the presence of a slight ptosis. The direct light reflex was absent but light directed into the dilated pupil produced a consensual pupillary constriction. Inconstant muscle palsies were produced by section of the oculomotor nerve. In two cats which had suffered section of the third cranial nerve, the size of the pupil varied slightly during the succeeding two or three days (Anderson, 6).

Five cats in which the oculomotor nerve was sectioned showed a temporary loss of the contralateral contact placing reactions. One animal which lived for five days was never able to walk. Since in all cases a beam of light directed into the dilated pupil produced constriction of the opposite sound pupil, functional integrity of the ocular reflex centers may be assumed.

The normally innervated cat eye was but little affected by the intravenous injection of 5 mgm. of acetylcholine per kilogram of body weight. Under the special condition of exposure to darkness, however, the pupil showed a slight dilatation in response to the drug (fig. 1, photographs). In general these results agree with the work of other investigators which indicates that the intravenous injection of small doses of acetylcholine is without effect (8), whereas the injection of large doses produces variable responses, none of which appear to be very great (9). Likewise, the instillation of two drops of 5 per cent acetylcholine into the conjunctival sac of the normal cat eye fails to induce pupillary constriction (4). In the animals in which a single operation was performed the pupil on the unoperated side served as the control.

Within 24 hours after section of the third cranial nerve or the removal of the ciliary ganglion, the intravenous injection of acetylcholine produced a constriction of the operated pupil. Indeed, the injection induced so great a constriction of the extremely dilated pupil that it became smaller

than the contralateral control pupil (fig. 1, photographs). This is an example of the "paradoxical pupillary constriction" of Anderson (6). The magnitude of the response to acetylcholine was much greater by the fifth day and was maintained at this level until about the eighteenth day, when it began to diminish. It reached a steady minimum in approximately thirty-five days. Figure 1 illustrates this phenomenon in a cat which had suffered section of the right oculomotor nerve. Similar pupillary

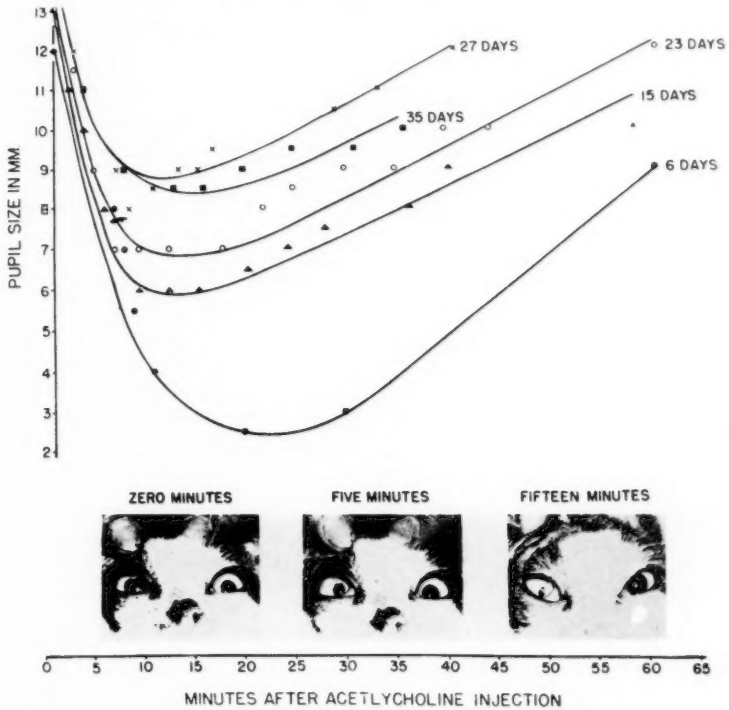

Fig. 1. The effect of intravenously injected acetylcholine (5 mgm. per kgm.) upon the size of the right pupil 6, 15, 23, 27 and 35 days after intracranial section of the right oculomotor nerve. Cat 1. The photographs refer to the six-day curve.

responses to acetylcholine after ciliary ganglionectomy are shown in figure 2. Constriction of the pupil on the side of the lesion was observed two to three minutes after injection. The constriction became maximal within seven to thirty minutes and gradually decreased during the succeeding half-hour. Pupillary constriction produced by acetylcholine injection, persisting long after the drug must have disappeared from the circulation, has been reported by Bender and Weinstein (8). It is important to note

that in our experiments the injection of acetylcholine produced an identical miosis whether the lesion interrupted pre- or post-ganglionic parasympathetic fibers.

To demonstrate that the diminished response to injected acetylcholine was related to the time which had elapsed since operation, bilateral ciliary ganglionectomy (2 cats) was carried out in two stages. Figure 3 shows that six days after the excision of the left ciliary ganglion and thirty-two days after the removal of the right ciliary ganglion the intravenous injection of 5 mgm. of acetylcholine per kilogram of body weight produced a

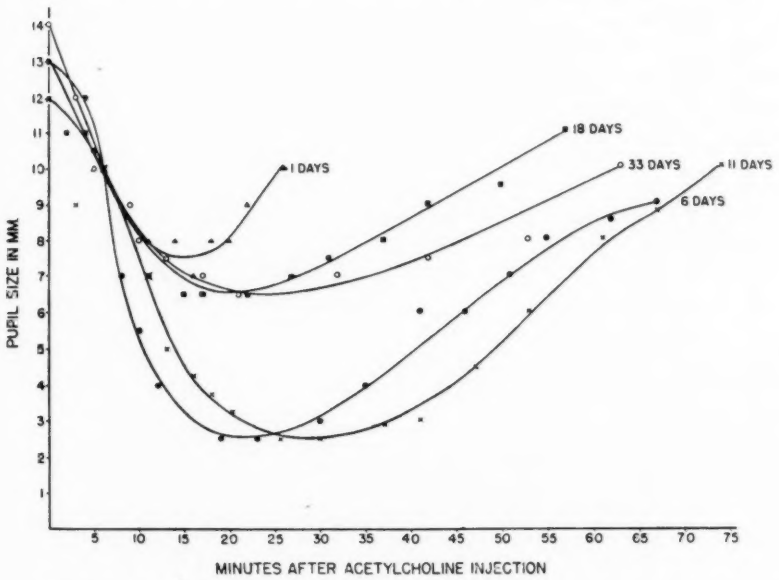

Fig. 2. The effect of intravenously injected acetylcholine (5 mgm. per kgm.) upon the size of the right pupil 1, 6, 11, 18 and 33 days after excision of the right ciliary ganglion. Cat 2.

constriction of both pupils. The more recently denervated sphincter showed a greater constriction than did that of the other side. The pupillary constriction curve produced by acetylcholine injection six days after excision of the left ciliary ganglion compares nicely with that obtained six days after removal of the right ciliary ganglion (compare figs. 2 and 3). These findings indicate that the time between the removal of the ciliary ganglion and the injection of acetylcholine constitutes an important factor in the magnitude of the pupillary response.

At various times after operation physostigmine was instilled into the

eyes of all of our animals. Two drops of a 1 per cent solution of this drug instilled into the conjunctival sac of the decentralized eyes produced the expected constriction (10). Several days after excision of the ciliary ganglion physostigmine was without effect (5, 10, and others). Instillation of physostigmine followed twenty minutes later by acetylcholine injection, in a cat sixty-two days after the removal of the left ciliary ganglion, induced

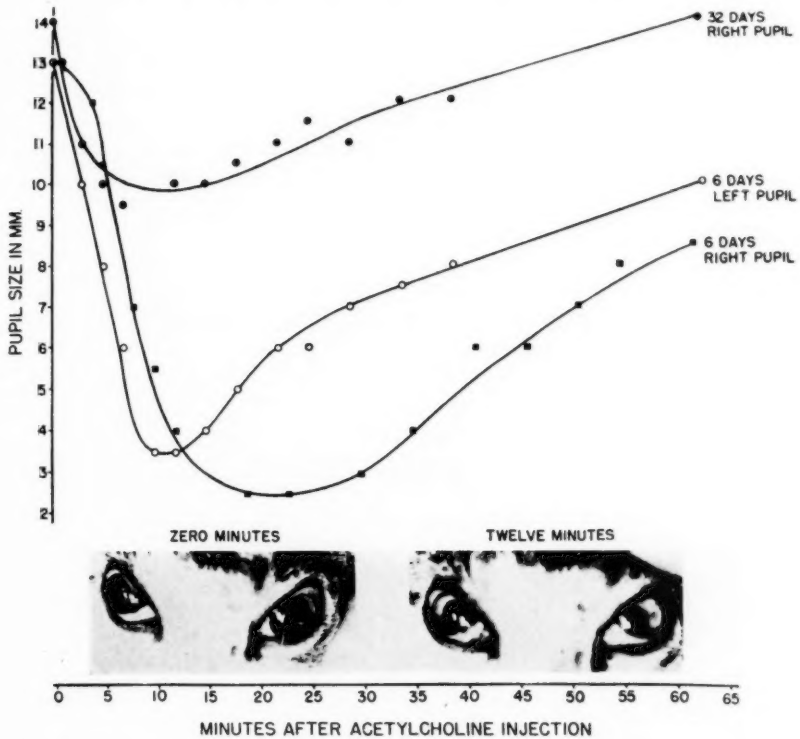

Fig. 3. The effect of intravenously injected acetylcholine (5 mgm. per kgm.) upon the size of the pupils 32 days after excision of the right ciliary ganglion and 6 days after removal of the left ciliary ganglion. The response of the right pupil 6 days after right ciliary ganglionectomy is included for comparison. Cat 2.

a constriction of the left pupil comparable with that which had been present eight days after operation (fig. 4). In this cat the reaction to physostigmine alone was negative, and the acetylcholine tests twenty-two and thirty-eight days after parasympathetic denervation showed that the pupillary response had diminished. The smaller pupillary response to acetylcholine, shown by the right eye of cat 2 (fig. 3), was increased when

physostigmine instillation preceded acetylcholine injection. Under these conditions the magnitude of the constrictions of both pupils was equal to that shown by the right eye in figure 3. To prevent the rapid destruction of acetylcholine by choline esterase, Shen and Cannon (4) instilled eserine in most of their experiments. Had this procedure been used

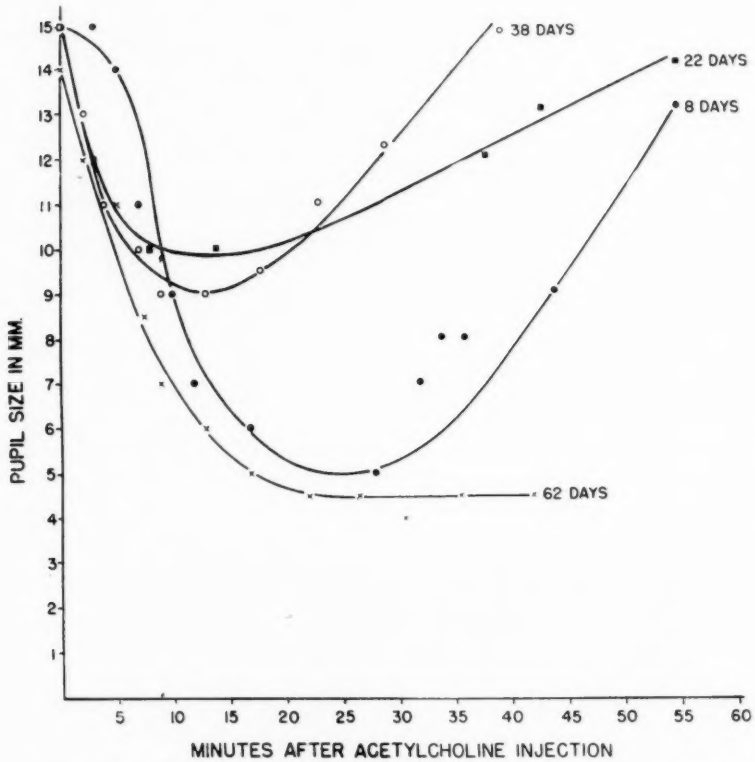

Fig. 4. The response of the left pupil to intravenously injected acetylcholine (5 mgm. per kgm.) 8, 22 and 38 days after left ciliary ganglionectomy. At 62 days 2 drops of 1 per cent physostigmine were instilled into the left eye followed 20 minutes later by the intravenous injection of acetylcholine (2.5 mgm. per kgm.). Physostigmine instillation alone was without effect. Cat 3.

routinely in our study the pupillary loss of sensitization to acetylcholine would have been missed.

Between two and three months after the excision of the ciliary ganglion the instillation of physostigmine alone into the conjunctival sac induced pupillary constriction. This observation confirms that of Anderson (10)

who noted a positive reaction in cats two months after ciliary ganglionectomy. According to Anderson the regenerating fibers come not only from the preganglionic stump, but also from motor branches of the oculomotor nerve to the extrinsic ocular muscles. This is understandable on the basis of the general rule that cholinergic fibers are interchangeable functionally with other cholinergic fibers (11). The accessory ciliary ganglia (10, 12) may also serve as a source of regenerating fibers. In agreement with Anderson, a positive reaction to physostigmine was observed at a time when no return of the light reflexes had occurred. It must be noted that the reaction to physostigmine was negative during the period of diminishing sensitivity to acetylcholine.

The rate of regeneration following section of the oculomotor nerve was highly variable. In one cat the pupil on the side of the lesion became less dilated, and twenty-nine days after operation it responded to light. Similar phenomena were shown by a second animal on the forty-seventh day. Two other cats which survived for long periods had shown no signs of regeneration at the end of fifty-nine and sixty-five days, respectively. These observations, confirming earlier studies of oculomotor nerve regeneration (6, 10), indicate that functional regrowth of the severed nerve fibers had not taken place during the period of diminished sensitivity to acetylcholine.

Marked systemic reactions were observed immediately after the intravenous injection of acetylcholine. The animal usually attempted to run, then fell upon its side in clonic and tonic convulsions. In some cases a few seconds of artificial respiration was applied; within five minutes the cat had recovered sufficiently to sit up. Profuse salivation, lachrymation, sweating of the foot pads, and dyspnea were present for about ten minutes. Urination and defecation occurred in certain animals. The systemic effects of acetylcholine injection were of greater intensity when instillation of physostigmine preceded the injection. Indeed, the injection of 5 mgm. of acetylcholine per kilogram of body weight killed one cat although this dose had been tolerated several times in the absence of physostigmine.

Discussion. Following the intravenous injection of adequate amounts of acetylcholine, contraction of the decentralized and denervated sphincter of the iris occurred. The secondary dilatation of the pupil induced by acetylcholine injection which has been reported by Bender and Weinstein (8) was not observed. Since these authors worked with cats having completely denervated pupils, it must be supposed that in the presence of sympathetic innervation secondary dilatation does not occur.

No apparent difference in the responses of the pupil to acetylcholine after pre- or post-ganglionic interruption of the parasympathetic fibers was observed. This is not surprising, for the post-ganglionic as well as the pre-ganglionic parasympathetic fibers are regarded as cholinergic (11).

The results of this study indicate that pupillary sensitization to the intravenous injection of acetylcholine decreases in about three weeks. That a systemic tolerance to the drug was not present is shown by the experiments in which the ciliary ganglia were removed at different times. Under these circumstances the more recently denervated sphincter showed a greater contraction which, however, followed the general contraction curve that the contralateral pupil had displayed previously. A second possible explanation is that excision of the ciliary ganglion might have injured the ocular sympathetic pathway in the orbit, and produced sensitization of the pupil to adrenin. Adrenin is known to be released by the direct action of intravenously injected acetylcholine upon the cells of the adrenal medulla (13, and others). This action, which might antagonize the pupillary response to acetylcholine, could account for the decrease in the observed parasympathetic sensitization. Such an explanation was excluded by demonstrating that the nictitating membrane of the eye from which the ciliary ganglion had been removed was not sensitized to injected adrenalin. This result agrees with the statement that the ocular sympathetic pathway does not pass through the ciliary ganglion in the cat (6, 12). That functional regeneration is not implicated was shown by the absence of the light reflexes, by the continued dilatation of the pupil, and, in the cats which had suffered ciliary ganglionectomy, by the negative responses to instilled physostigmine.

The acetylcholine sensitization described in this investigation could be explained by assuming that the interruption of parasympathetic fibers decreases the activity of the choline esterase system. Such an assumption is supported by the reports that section of the autonomic innervation of certain tissues decreases the choline esterase content (14, 15) and activity (15, 16). Moreover, ciliary ganglionectomy is said to result in the disappearance of acetylcholine from the iris and ciliary body (17). The experiments, in which physostigmine was first instilled into the eye, suggest that the diminished response to acetylcholine injection, occurring, as it did, three weeks or more after excision of the ciliary ganglion, is related to an increased activity of the esterase system. Under these circumstances pupils which had shown decreased responses to acetylcholine alone, now gave a maximal contraction to the injection of even small amounts of the drug.

In view of the above study, it is of interest to note that Pierce and Gregersen (18) injected 100 mgm. of acetylcholine into dogs two months after section of the chorda tympani and found no evidence of sensitization, as measured by the salivary flow. These authors suggested that sensitization may have been present at an earlier period.

On the basis of choline sensitization, Scheie (19) has placed the site of the disturbance of Adie's syndrome in the parasympathetic system at the

ciliary ganglion or peripheral to it. Our results, which show that acetylcholine sensitization follows section of the oculomotor nerve, do not support this conception.

SUMMARY

1. The normal pupil of the cat reacts only slightly to the intravenous injection of 5 mgm. of acetylcholine per kilogram of body weight.

2. Removal of the ciliary ganglion or intracranial section of the oculomotor nerve results in a marked acetylcholine sensitization of the iris sphincter. The magnitude of this sensitization is approximately the same whether the sphincter is denervated or decentralized.

3. Sensitization of the iris sphincter reached a maximum in about five days and similar curves could be obtained until about the eighteenth day. After this time the responses decreased until they reached a steady minimum within approximately thirty-five days.

4. The recovery of acetylcholine sensitization following the instillation of physostigmine suggests that the loss of sensitization is associated with an increased choline esterase activity.

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COMPARATIVE EFFECTS OF PROGESTERONE AND CORTICO-ADRENAL EXTRACTS ON NORMAL, ADRENALECTOMIZED AND OTHER ANIMALS[1]

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The work of Gaunt (1937) *et al.* (1938, 1939) has apparently established the ability of progesterone to maintain the life of the adrenalectomized ferret and has served to revive interest in the possibility of a direct interrelationship between cortico-adrenal and ovarian secretion. Stewart (1913) first called attention to what he considered an unusual prolongation of the life-span of pregnant cats following adrenal removal, and later Stewart and Rogoff (1925), working with dogs, reported that pregnant animals survived adrenalectomy until the end of gestation. They suggested that the corpora lutea might be responsible for the unusually long survival periods observed. The writer (1928) was unable to substantiate these claims in experiments on an extended series of adrenalectomized pregnant and lactating cats.

Results on the rat. In the present study, comparisons were first made of the effects of cortico-adrenal extract and progesterone[2] on the carbohydrate metabolism of normal, fasted, adrenalectomized and hypophysectomized rats. Having long maintained that cortico-adrenal secretion is intimately concerned with carbohydrate metabolism (Corey, 1927, 1935; Corey and Britton, 1937, 1939), it was felt that such a comparison was essential to an evaluation of progesterone in the rôle of a substitute for cortico-adrenal secretion. The results are briefly presented in table 1. Figure 1 shows the results graphically.

Both table and figure indicate that progesterone, in the amount administered, did not influence carbohydrate metabolism in the rat. This was in distinct contrast to the marked rise in blood sugar and hepatic glycogenesis observed following cortico-adrenal extract administration.

[1] Grateful acknowledgment is made of aid received from the Committee on Research in Endocrinology of the National Research Council.

[2] We wish to thank the Schering Corporation, and particularly Dr. W. R. Bond of their Medical Research Staff, for supplying the considerable quantities of progesterone ("Proluton"; crystalline, synthetic progesterone) employed in this study.

TABLE 1

Effects of cortico-adrenal extract and progesterone on carbohydrate metabolism in the rat

NUMBER OF RATS	CONDITION OF ANIMALS	TREATMENT	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN
			mgm. per cent	*grams per cent*	*grams per cent*
A. Animals in which all blood and tissue samples were taken 6 hrs. after the injections were made					
3	Normal, fully fed	5 cc. cortico-adrenal extract	96	2.72	0.32
8	Normal, fully fed	5 mgm. progesterone	95	2.14	0.33
2	Normal, fully fed	1 cc. 0.9% NaCl	96	2.21	0.29
4	Normal, fasted for 24 hrs.	5 cc. cortico-adrenal extract	82	0.52	0.30
8	Normal, fasted for 24 hrs.	5 mgm. progesterone	66	0.09	0.24
2	Normal, fasted for 24 hrs.	1 cc. 0.9% NaCl	64	0.08	0.26
8	Adrenalectomized 4 days previous to experiment	5 cc. cortico-adrenal extract	90	0.71	0.30
7	Adrenalectomized 4 days previous to experiment	5 mgm. progesterone	64	0.24	0.31
2	Adrenalectomized 4 days previous to experiment	1 cc. 0.9% NaCl	66	0.21	0.33
B. Injections made every hour for 24 hrs., when blood and tissue samples were taken					
2	Hypophysectomized 7 days previous to experiment	1 cc. extract	98	0.72	0.30
4	Hypophysectomized 7 days previous to experiment	1 mgm. progesterone	76	0.15	0.16
2	Hypophysectomized 7 days previous to experiment	1 cc. 0.9% NaCl	75	0.13	0.15

C. Blood-sugar samples only

			B. S. (INITIAL)	B. S. (2 HRS.)	B. S. (4 HRS.)	B. S. (6 HRS.)
			mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
3	Normal, fasted for 24 hrs.	5 cc. cortico-adrenal extract	74	99	92	73
3	Normal, fasted for 24 hrs.	5 mgm. progesterone	72	68	73	66

It was concluded that progesterone does not act similarly to cortico-adrenal extract in the rat since, unlike cortical preparations, it was wholly without carbohydrate influence in that animal.

Results on the cat. In a study of the action of progesterone in the cat, 21 animals were adrenalectomized and progesterone injection was begun either at once, or after symptoms of adrenal insufficiency had become plainly evident.

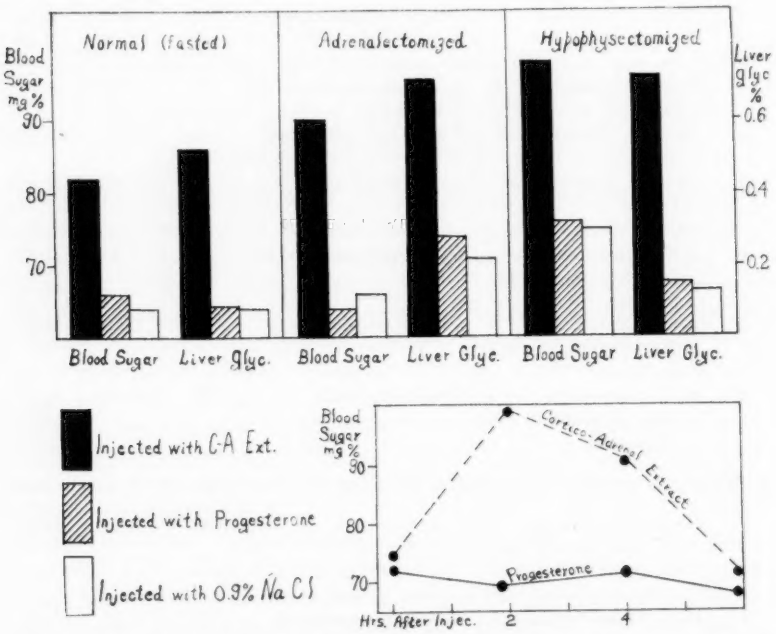

Fig. 1. Comparative effects of cortico-adrenal extract and progesterone on the blood sugar and liver glycogen levels of rats under various conditions.

Three male animals evinced symptoms of adrenal insufficiency (weakness, anorexia, ataxic gait) following bilateral adrenalectomy after an average of 4.5 days. Progesterone (5 mgm. twice daily) was then given until the animals were restored to an apparently normal condition, when the dosage was reduced to 5 or even 2 mgm. per day.

Two of the animals were maintained on progesterone for 21 days, this period being considered sufficiently long to indicate definite life-maintaining potency; also the large amounts of progesterone demanded by such experiments precluded more prolonged study. One animal was sacrificed while in apparent good health and the blood sugar, serum sodium, chloride,

potassium and urea levels were determined, as well as the liver, skeletal and cardiac muscle glycogen concentrations. All the analyses yielded results well within the normal range. The second animal was allowed to develop symptoms of adrenal insufficiency before progesterone administration was begun; it showed a definitely favorable reaction to the injections and was in good health after three weeks' treatment. Blood and tissue samples were taken 10 days after stopping injections, when the cat exhibited signs of profound adrenal insufficiency. Severe carbohydrate depletion, lowered sodium and chloride levels with uremia, and a high serum potassium concentration were present, i.e., the usual conditions found in cats suffering severe cortico-adrenal insufficiency. A protocol of observations on this animal, appended below, demonstrates the life-maintaining action of progesterone together with the glycemic response to injections obtained in this instance.

Protocol, cat 2. Male, weight 2.59 kilos. March 1, 1939, adrenalectomized (blood sugar 81 mgm. per cent).

March 6, 9:55 a.m. Weak, staggers. B.S. 66 mgm. 3:45 p.m. Much weaker. 10:00 p.m. Given 5 mgm. progesterone. Treatment begun (10 mgm. daily).

March 7 to 11, cat in excellent condition; appetite good; active. Average B. S. for this period 76 mgm. March 11, dosage reduced to 5 mgm. daily.

March 11 to 20, condition still good. Apparently normal. Average B. S. for this period, 74 mgm. March 20, condition unchanged; B. S. 78 mgm. Weight 3.00 kilos. Injections of progesterone stopped.

March 21 to 27, condition still good, although appetite poor on occasions. Average B. S. for this period 71 mgm.

March 28, 8:30 a.m. Condition suddenly became much worse; very weak, staggered when compelled to walk; progesterone dosage increased to 20 mgm. daily.

March 29, 8:30 a.m. Condition no better. 3:30 p.m. Convulsions, prostrate, dyspneic respiration. Animal sacrificed. Terminal blood and tissue analyses made: B. S. 45 mgm. per cent. Liver glycogen, 0.11 gram per cent; muscle glycogen, 0.12 gram; cardiac glycogen, 0.27 gram; serum sodium, 120 mE/L.; chloride, 86 mE/L.; potassium, 17 mE/L.; urea, 144 mE/L.

The third male cat showed definite insufficiency symptoms 3½ days following operation. Recovery was effected with progesterone as in the above two cases, and the cat was maintained for 13 days in apparently good health. At this time symptoms of insufficiency recurred, in spite of greatly increased progesterone dosage, and the animal was sacrificed 18 days after operation. Blood-chemical findings revealed conditions indicative of a mild adrenal insufficiency.

It should be added that in all 3 instances above, insufficiency and recovery were well reflected in the frequent blood sugar determinations made throughout the experiments. Moreover, it may be noted that in all cases a definite life-maintaining action on the part of progesterone was demonstrated.

With the hope of discovering whether testosterone might be in part responsible for the extended survival periods described above, 2 male cats were castrated, and, after a period of three weeks, were adrenalectomized. These cats survived during progesterone injection for an average of 21 days. Both animals succumbed, however, during treatment, in spite of heavy progesterone dosage. On the other hand, a definitely beneficial effect of the progesterone was demonstrated, since their average survival period was roughly 4 times that of untreated adrenalectomized cats in this laboratory. It was concluded that castrated male cats may survive adrenalectomy for rather extended periods, although not indefinitely, when injected with progesterone.

Ten female cats were adrenalectomized and experimentation continued with progesterone treatment as above. The average survival period for these animals was 4 days, or approximately the survival expectancy for untreated adrenalectomized cats. In no instance was any ameliorative effect of the progesterone observed, in spite of daily administration of 10 or even 20 mgm. of the preparation. No significant effect of progesterone on the blood sugar was evident at any time and low terminal values were observed in all instances. It was concluded that progesterone has no demonstrable effect on the life-span of adrenalectomized female cats, in marked contrast to its beneficial influence on males. This conclusion had been reached in earlier experiments of a similar nature, and a brief preliminary report was published (1939).

It now appeared that estrone in the blood of the female cats might be responsible for the failure of progesterone treatment in these experiments. Gaunt (1937), working with ferrets, had published results which might be so interpreted. Therefore 3 female cats were ovariectomized, and 3 weeks later when recovery from the initial surgery was complete, both adrenal glands were removed.

One of these animals was maintained in excellent condition for 22 days on a daily dosage of from 4 to 10 mgm. of progesterone. During this period the animal appeared normal in every way, but 4 days after withdrawal of the progesterone it died (26th day after adrenalectomy) with symptoms of acute adrenal insufficiency. Terminal blood and tissue samples were not obtained from this animal due to the rapidity with which the syndrome developed.

The second ovariectomized-adrenalectomized cat refused food from the day of operation and was given 10 cc. of cortico-adrenal extract together with 10 mgm. of progesterone daily for the first 2 post-operative days. Appetite returned and the animal was maintained for 24 days on progesterone alone (total survival, 26 days). It was sacrificed while in good condition, and the blood chemistry showed values well within normal limits. Two relapses during treatment which necessitated increasing the

dosage of progesterone attested to the completeness of adrenal removal as did the autopsy findings.

The third animal reacted in much the same manner as the above, refusing food from the day of operation. Ten cubic centimeters of cortico-adrenal extract plus 10 mgm. of progesterone restored appetite within 2 days and the animal was then maintained on the progesterone alone for a total of 19 days, the dosage being gradually reduced to 5 mgm. per day. Treatment was then stopped and the animal died three days later, exhibiting symptoms of severe adrenal insufficiency. Terminal blood-chemical findings showed the markedly lowered glycemic levels and electrolyte shifts typical of cortico-adrenal hormone depletion.

It was concluded that ovariectomized-adrenalectomized cats may be maintained alive for periods considerably in excess of the usual life-span of similarly operated, untreated animals.

With the assumption that normal luteal secretion coupled with progesterone injection might aid in maintaining life, a further short series of experiments was undertaken. Three pregnant female cats were adrenalectomized and thereafter maintained on progesterone (10 mgm. daily). These animals survived for an average of 13 days. Slight elevation of the blood glucose levels followed the injections, but symptoms of insufficiency occurred precipitately in 2 instances. The third animal was sacrificed 17 days after operation, when marked symptoms of insufficiency appeared in spite of increased progesterone dosage. Blood and tissue chemical values were found to be characteristic of adrenal insufficiency.

It appeared that the luteal factor in the pregnant cat had an ameliorative effect on the development of cortico-adrenal insufficiency, but the effect was slight; even though coupled with injections of progesterone, luteal secretion was incapable of maintaining the life of the adrenalectomized females cats for prolonged periods. The presence of estrone as a factor in precipitating insufficiency symptoms in these animals is suggested.

Discussion. The present experiments demonstrate that progesterone does not maintain blood glucose and liver glycogen in normal, adrenalectomized or hypophysectomized rats. Altogether 30 cases were studied. This is in sharp contrast to the action of cortico-adrenal extract, which was markedly effective in all (20) cases observed. Gaunt, Remington and Edelman (1939) have reported a similar failure of progesterone to affect carbohydrate metabolism in normal (3) rats; they found on the other hand that the liver glycogen levels of fasting ferrets (5) was elevated following progesterone injection.

The life-maintaining effect of progesterone in adrenalectomized (otherwise normal) male cats is demonstrated; thus the onset of symptoms of adrenal insufficiency may be postponed for extended periods (up to 3 weeks) by the luteal hormone. Although undoubted evidence of an ameli-

orative effect was demonstrated in the cat, it is doubtful whether "indefinite" survival of such adrenalectomized animals could be attained by the use of progesterone, even in massive doses. Thus cat 3, although restored with progesterone from symptoms of insufficiency, could not be maintained in health and was sacrificed on the 18th post-operative day in acute insufficiency. Cats 20 and 21 (castrated-adrenalectomized males) also failed to respond favorably to large (10 mgm.) daily injections of the hormone, and developed symptoms of severe insufficiency 18 and 19 days after operation. Further progesterone injections were, moreover, without apparent benefit. One died suddenly and the other was utilized for samples when no improvement in its condition was evident.

On the basis of results obtained with adrenalectomized, non-pregnant females, one is led to the conclusion that progesterone was without beneficial effect in such animals. Since all progesterone-treated male, as well as ovariectomized and pregnant female cats, survived adrenalectomy for rather protracted periods (av. 20 days), it appears that the presence of estrone may explain the non-beneficial effect of progesterone in adrenalectomized (but otherwise normal) female animals.

Aside from theoretical considerations, it is apparent that progesterone cannot be looked upon as a complete substitute for the cortico-adrenal hormone, although recovery from the earlier symptoms of insufficiency and a significantly extended life-span may be demonstrated following adrenalectomy in the cat.

It was noteworthy throughout the study on the cat that an animal's condition was closely related to the glycemic and hepatic glycogen levels encountered. Thus all animals exhibited carbohydrate values within the normal range when in good condition, whether maintained on progesterone or a short course of cortico-adrenal extract to promote post-operative recovery. The ameliorative effects of progesterone on adrenal insufficiency may therefore be possibly attributed to its influence on carbohydrate metabolism.

SUMMARY

Progesterone injection in fasting, adrenalectomized or hypophysectomized rats has no significant influence on glycemic or glycogen levels. The effects of cortico-adrenal extract are in contrast markedly effective under all of the conditions noted.

The life-span of adrenalectomized male, as well as adrenalectomized pregnant or ovariectomized female cats, is considerably lengthened—from 4 to 5 times the usual life expectancy—by progesterone administration. Moreover, recovery from the symptoms of adrenal insufficiency may be effected by progesterone in such animals.

It is doubtful whether survival of the adrenalectomized cat may be indefinitely prolonged by the administration of progesterone.

The condition of adrenalectomized cats, whether treated with progesterone or cortico-adrenal extracts, was related to the glycemic and tissue glycogen levels observed throughout the study.

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COMPARATIVE STUDIES OF THE METABOLISM OF THE BRAIN OF INFANT AND ADULT DOGS[1]

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Hughlings Jackson made the suggestion that the nervous system is organized in such a way that the primitive reactions of the older parts are prevented from dominating behavior by inhibiting influences emanating from the higher levels. Thus, removal of some rostral part of the cerebrospinal axis may result in release phenomena. Examples of such release are seen in the decorticate and the decerebrate preparations. Similar phenomena are observed during hypoglycemia (1, 2) and anoxia (3) when the earliest effects reveal depression of the cortex and the basal ganglia and hypothalamus are released. Then the basal ganglia and hypothalamus are depressed while the midbrain becomes dominant. The midbrain is inhibited in turn when the subject exhibits the release phenomenon of decerebration (1, 2, 3).

Hoagland et al. (4) have noted that during marked hypoglycemia the cortical rhythms of the dog disappear before those of the thalamus. By producing cerebral anoxia, Heymans and others (5) have shown that the higher more newly developed parts of the adult brain are less resistant than the older regions. Sugar and Gerard (6) have reported similar results using the electroencephalograph. It has been previously demonstrated that the various parts of the ox brain (7) and rat brain (8) consume oxygen at different rates. The suggestion has, therefore, been made that the order of appearance of the release phenomena of anoxia and hypoglycemia and the alterations in electrical activity may be related to the different metabolic rates of the various parts of the brain. In order to obtain more information on this subject we have determined the oxygen consumption of the various parts of the brain of the adult dog. Additional light may be thrown on this subject by investigating the developing central nervous system. For this reason similar studies were made on the various parts of the brain of growing puppies.

METHOD. The present observations were obtained on minced cerebral tissue of puppy and adult dog placed in the Warburg respirometer and

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suspended in a saline solution buffered with phosphate at pH 7.4, with glucose as substrate. The brain of both the young and adult dogs was dissected into the following parts: cortex, thalamus, cerebellum, medulla, midbrain and caudate nucleus. A few observations of the gray matter of the cord of puppies were also made, and in all determinations an effort was made to eliminate the white matter. Puppies from one week to seven weeks of age were studied and the results obtained for each region for each week of post natal life averaged. The parts of the brain were weighed before and after drying at 105°C. and the differences represent the aqueous contents.

RESULTS. The results in the adult brain reveal different rates of metabolism in the various cerebral parts (table 1 and fig. 1). The caudate nucleus possesses the highest oxygen consumption and the medulla the lowest. The cerebral cortex, cerebellum, thalamus and midbrain are

TABLE 1

The oxygen uptake mm³ per 100 mgm. moist tissue per hour at different ages of dogs

PARTS OF THE BRAIN	1ST WEEK		2ND WEEK		3RD WEEK		5-7TH WEEK		ADULT	
	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages
Cortex	47	61	26	64	37	68	8	121	25	116
Caudate nucleus	9	73	5	88	6	96	2	139	20	136
Midbrain	16	91	14	99	13	111	6	128	10	92
Medulla	14	96	15	101	19	103	6	85	16	69
Thalamus	25	76	15	93	14	97	6	124	18	101
Cerebellum	14	79	19	85	25	87	9	95	22	107
Spinal cord	13	81	7	82	4	93			2	50

intermediate with values of somewhat the same order. The oxygen consumption of these regions of the infant brain one week of age is strikingly different from the adult. In the first place the caudate nucleus and the cortex present values which are definitely less than the corresponding parts of the adult brain. The medulla on the other hand exhibits an oxygen consumption almost twice that of the adult tissue. The few observations of the cord also reveal a higher level in the newborn. The second week of life is characterized by a slight, though significant, increase in the oxygen consumption of practically all parts of the infant central nervous system. The same order as that for the first week of life nevertheless persists with the medulla and cord highest and the cortex lowest. Caudate nucleus and cerebellum possess intermediate rates similar to the observations of the first week.

During the third week of post natal life the most outstanding phenomenon is that the midbrain changes its relative position and assumes with

the medulla the highest oxygen consumption. The cortex still remains the lowest, while the cerebellum, thalamus, and caudate nucleus are significantly higher than the medulla. From the fifth to the seventh week the oxygen consumption of all parts with the exception of the medulla is definitely higher than the corresponding values for the first week of life. The caudate nucleus possesses by far the highest oxygen consumption at this time. The thalamus and midbrain are second in order. The cortex exhibits an oxygen consumption distinctly higher than either the cere-

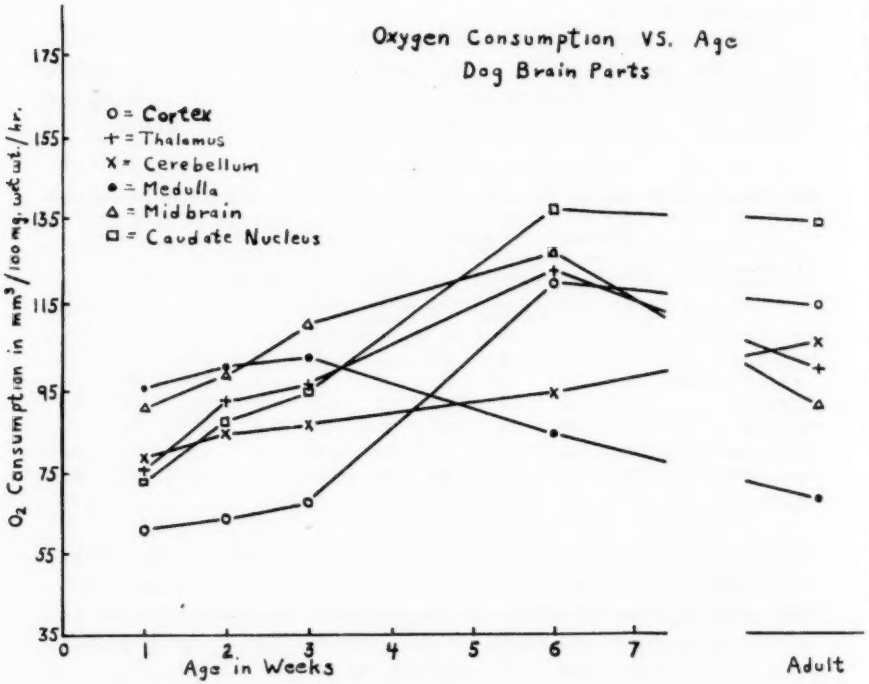

Fig. 1

bellum or the medulla. The latter has at this time an oxygen uptake lower than any of the other parts, lower than the values observed during the first week of life. The observations on the percentages of the aqueous contents of the brain disclose a gradual increase of solids of all the parts studied (table 2). At each age the lower parts are characterized by greater concentrations of solids.

Discussion. In general, these results on dogs are in agreement with previous ones observed on rats (8) insofar that the metabolic rate of the

brain of the newborn is lower than that of the adult. Throughout the first seven weeks of life the caudate nucleus, the cerebral cortex, the thalamus, cerebellum, and medulla exhibit increases in metabolism. A review of table 2 shows a simultaneous rise in solids. These results are not numerous, but they agree with others on rats (9) indicating a greater percentage of dry weight in the brain with increasing age. Whatever determines metabolic rate, whether it is total cellular surface, active respiring surfaces, concentrations of protein or enzymes, probably varies at least partially with total solids (10). In the first week of life the solids of the different regions of the brain are in the same order as their metabolic rates. Thus, the lower parts of the brain contain less water at this time. This relationship, however, does not always hold. Despite a continued decrease in fluid contents the adult dog exhibits a decline of the metabolic rates of some parts of the brain in comparison with that of the puppy in the fifth

TABLE 2

Water contents (per cent) of puppy and adult dog brain

PARTS OF THE BRAIN	1ST WEEK		2ND WEEK		3RD WEEK		5-7TH WEEK		ADULT	
	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages	Number of observations	Averages
Cortex	27	88.1	14	88.0	5	88.6	5	84.0	6	82.8
Caudate nucleus	2	91.0			1	87.0	2	83.0	3	79.7
Midbrain	5	85.0	4	86.5	2	85.0	1	79.0		
Medulla	5	84.8	4	84.5	1	82.0	3	78.0	1	72.0
Thalamus	10	86.9	9	86.6	3	87.3	2	82.5	1	70.0
Cerebellum	5	87.2	8	85.1	4	84.5	3	82.0	3	83.2

to the seventh week of life. In the adult it is more difficult to separate gray from white matter and it is possible that the apparent decreases in the metabolic rate may be ascribed to the presence of a certain amount of contaminating white matter with its lower metabolism. This applies particularly to the observations on medulla and cord. If the precursor of the white matter in the brain of the newborn either possesses a relatively higher metabolism or represents a smaller percentage of the total tissue, before myelinization takes place, contamination with this material would exert a less important effect on respiration from a quantitative point of view.

The present results have been calculated on a moist basis of 100 mgm. of tissue and, therefore, may approximate the metabolism of the various parts of the brain *in situ* (11). It is, therefore, interesting that a metabolic progression which advances rostrally in the central nervous system is disclosed. The lower parts are most active at birth and as development

continues the wave of metabolism proceeds forward so that the lower portions of the central nervous system are surpassed by the anatomically higher and phylogenetically more recent regions. Other observations in the literature are in accord with this conception. It is generally accepted that early activities of the fetus involve chiefly the lower parts of the neuraxis. Hooker (12) noted that human fetuses, $8\frac{1}{2}$ to 14 weeks old, respond to stimuli only by spinal and medullary reflexes. Even the central nervous system of the newborn operates on the basal ganglion level as evidenced by the sucking and forced grasping reflexes. Cortical functions are practically absent in the newborn. This is in agreement with the low oxygen consumption of the cerebral cortex during this age of life.

Nachmansohn (13) found that choline esterase is present in high concentrations in the spinal cord of sheep fetuses at an early stage of gestation. At this time it is low in the brain centers where it subsequently rises rapidly before birth. Thus Nachmansohn observed increasing activity in the medulla early in gestation, but this activity diminished as myelinization progressed. On the other hand, the caudate nucleus which starts with a lower esterase activity than the medulla finally surpasses it. Thus the mechanisms for the conduction of the nerve impulse as well as for the delivery of energy are developed in the same phylogenetic order and permit the establishment of the functions of the corresponding parts of the central nervous system.

SUMMARY AND CONCLUSIONS

1. With the aid of the Warburg apparatus, determinations were made of the oxygen consumption of various parts of the brain of puppies and adult dogs. The respiratory metabolism of the cerebral tissues was found to be characteristic according to the part of the brain and to the age of the animal.

2. The oxygen consumption of the newborn dog is lower than that of the adult. In the growing puppy during the first seven weeks of life the increase in metabolic rate proceeds rostrally from medulla to caudate nucleus. In the adult brain the respiratory metabolism of the various cerebral parts are higher in the newer phylogenetic layers. These differential rates of metabolism may yield a basis for the order of the symptoms of hypoglycemia and acute anoxia.

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THE INHIBITION OF GASTRIC SECRETION BY THE INTRAVENOUS INJECTION OF CALCIUM SALTS

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The essential interest of this problem lies in the hypothesis, for which clinical and physiological evidence exists, that the parathyroid glands in some way, at present not understood, influence the function of the gastric glands.

Babkin, Komarov and Komarov (1, 2) have recently investigated the effects of activated ergosterol and parathyroid hormone on gastric secretion in dogs. In their paper the literature on the subject has been reviewed and, as they have stated, "the data concerning the effect of hyper- and hypocalcemia on the nervous and humoral regulatory mechanisms of the gastric glands are few and contradictory." Their results support the view that the physiological agents which lead to hypercalcemia have an inhibiting effect on gastric secretion—this effect appearing markedly during the nervous phase but only slightly during the chemical.

A change in the level of blood calcium caused by a disturbance of parathyroid function, or by parathormone given therapeutically or to normal animals, or by ergosterol, is accompanied by disturbances in the mechanisms by which these agents maintain normal blood calcium, and any change found in the gastric gland function may be due either to the changes in blood calcium or to the disturbances causing such changes acting directly on the glands. On the other hand, when calcium is injected into the blood the conditions are simplified and the effect of blood calcium *per se* may be studied. But in this case, too, we have to differentiate between injecting calcium as a therapeutic agent to combat a low blood calcium, and injecting it into normal animals, for in the former case we have the complicating factors mentioned above.

The inhibition of gastric secretion, to be described, occurred after the intravenous injection of calcium salts into normal dogs in acute experiments in which the gastric glands were stimulated by histamine or through the vagi with the induction current.

EXPERIMENTAL PROCEDURE. The dogs had access to water but were fasted for 18 hours before the experiment was begun. Throughout the experiment chloralose-urethane anesthesia was maintained and the ani-

mals were kept warm. The following operations were performed: tracheotomy, cannulation of the carotid artery and jugular vein, preparation of the vagi for sectioning later in the experiment, esophagus (in the neck) tied, duodenum tied off, stomach fistulated, urinary bladder cannulated. (In 5 experiments the secretion of the pylorus was collected separately through a cannula in the distal end, the body of the stomach having been separated from it by tying a grooved rubber stopper at the pyloric entrance.) The animal was then placed prone on the supporting frame and during a period of 1½ to 2 hours the resting secretion and excretion were collected. Towards the end of this period the vagi were cut.

When the vagi were stimulating agents for the secretion, rhythmic tetanic stimuli from the inductorium were delivered alternately for 7½ minutes to each nerve. When histamine (dihydrochloride) was used, 0.5 mgm. doses were injected subcutaneously at 15-, 30- or 45-minute intervals, depending on the amount necessary to maintain a constant, suitable rate of secretion. With this aim also in view, the strength of the current when the vagi were being used was regulated.

When the secretion remained constant for two or more 15-minute collection periods, the injection of either 5 per cent calcium lactate or 1.6 per cent calcium chloride was made (in most cases 13 mgm. Ca per kgm. body weight was the dose and cases in which fractions of this were given are indicated in the tables). In a few cases it was possible to give four successive injections with adequate recovery periods between each, without signs of failure of the gastric gland function; on the other hand, in some experiments complete data were obtained only for the first injection, the gastric gland function after this failing to maintain its initial responsiveness.

Blood samples for calcium were taken at infrequent intervals—the first sample after the injection, not before the inhibition had definitely set in. The Kramer-Tisdall (3) method, as modified by Clark and Collip (4), was used.

The blood flow through the stomach was determined by measuring with a stop-watch the time for 5 cc. of blood to flow into a syringe from a cannula placed in the gastro-splenic vein near the spleen, this organ having been cut off from the circulation and the vein clamped distally during the period of collection.

Results. 1. The degree of inhibition, the time after injection at which it was maximum, its duration and after-effects are summarized in table 1. In all, 8 injections of the calcium salts were made in 4 experiments in which the stimulus was through the vagi, and 21 injections in 13 experiments where histamine was used; of the latter, 9 were cases in which the pylorus was excluded.

In all cases inhibition occurred. The average reduction for the 1st

injection, equivalent to 13 mgm. Ca/kgm. body weight, ranged from 22.5 to 97.8 per cent (average, 74 per cent); for the 2nd injection, a fraction of the initial dose, it ranged from 42.0 to 99.2 per cent (average, 76 per cent). The significance ascribed to the fact that the 2nd dose, although as a rule a fraction of the 1st, had as great an effect, will be discussed.

The inhibition was maximum 45 minutes after the injection (variations from 30 to 67 min.) and had ended in 75 minutes (variations from 52 to 105 min.) on an average. Cases showing the slowest recovery were those after the 2nd injection, in some of which it was incomplete in 2 hours. In the cases in which the pylorus was excluded, the average reduction was

TABLE 1

Summary of experiments showing the average reduction in the rate of gastric secretion and the time of maximum inhibition and its duration, following the injection of calcium

STIMULUS	CALCIUM INJECTED PER KGM. BODY WEIGHT	NUMBER OF INJECTION	REDUCTION OF SECRETION (VARIATIONS IN BRACKETS)	INHIBITION, MINUTES AFTER Ca		NUMBER OF EXPERIMENTS
				Maximum	Duration	
	mgm.		*per cent*			
Vagus	13	1st	76.5 (44–96)	45	76	4
Vagus	4.3, 6.5	2nd	83.4 (74.4–92.5)	67	112†	2
Vagus	13	2nd	53.0 (42–64.3)	‡		2
Histamine	13	1st	83.4 (50–97.8)	43	90	6
Histamine	9	1st	40.5	30	60	1
Histamine	9, 9, 6.5, 6.5	2nd	80.9 (42–99.2)	49	110†	4
Histamine	13	3rd	92.2	45	105	1
Histamine*	13	1st and 2nd	61.9 (22.5–87.1)	45	52	4
Histamine*	6.5, 9, 10.4	1st	51.2 (41.2–68)	40	71	3
Histamine*	6.5, 8.6	2nd	65.7 (62.2–69.2)	45	90†	2

* Pyloric secretion excluded.

† Incomplete.

‡ Probably not reached before experiment ended.

less and the period of inhibition shorter than in the cases in which the total secretion was collected.

Following the period of inhibition an increase over the control volume of secretion was observed in 13 cases which were carried on over sufficiently long periods. This increase amounted to 47 per cent (variations 5 per cent to 103 per cent) and occurred $1\frac{3}{4}$ hours (variations $\frac{3}{4}$ to 3 hrs.) after the injection.

2. The comparative effects of sodium lactate and calcium lactate are shown in figure 1, representing one of 4 experiments, of which this one, in which it was possible to give 3 or 4 successive injections, provided us with the best conditions for a comparison. The sodium lactate caused no inhibition in rate of secretion but rather the reverse.

3. With respect to the mechanism of inhibition, an osmotic pressure change was eliminated as an important factor by using solutions isotonic with blood. In addition the changes in urinary excretion were only very slight; and the freezing-point of the blood, determined at the critical periods of a typical experiment, showed no change which could be considered significant (fig. 2). That circulatory changes were not important causal factors was the conclusion from results of experiments in which the maximum inhibition of secretion was seen long after the blood pressure (table 2) and blood flow through the stomach (table 3) had returned to normal. The experiment given in table 3 is one of two in which the blood flow through the stomach was measured. The gastric secretion was not registered.

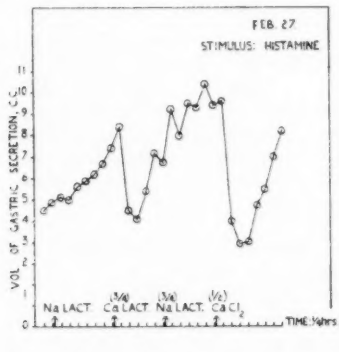

Fig. 1. Left. The comparative effects of calcium lactate, calcium chloride and sodium lactate on gastric secretory rate.

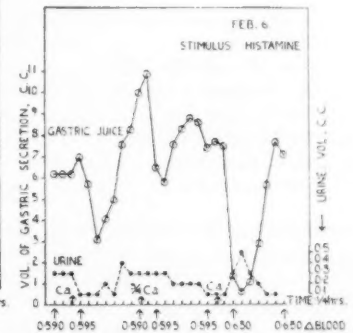

Fig. 2. Right. Effect of calcium lactate on volume of gastric secretion and on osmotic pressure of blood.

4. The blood calcium results (table 4) are taken from all experiments and give a general picture of the changing level. Figures taken from an experiment of Smith and Sternberger (5) are complementary to ours and make it appear probable that the maximum calcium of 16 to 17 mgm. per cent was reached a few minutes after the injection.

Discussion. In these experiments the calcium salts inhibited both the nervous and the chemical type of secretion. From this it would be inferred that the effect is not a specific interference with the initiation or transmission of the nervous impulse, as appeared to be the case in the experiments of Babkin, Komarov and Komarov (1), especially those with ergosterol. However, in their experiments a much more profound disturbance involving the obscure processes controlling the blood calcium level must exist, in contrast to the conditions in mine where we are dealing simply with an excess of calcium, probably in the ionized form, which

merely may have to be excreted by one route or another, or stored. It is possible that the difference between the ergosterol and the parathormone effects found by Babkin *et al.* may be due to a difference in the action these agents have on the balance of the calcium fractions, because as was shown by Mr. Schiffrin in this laboratory (see Babkin, 2) parathormone produces a more marked inhibition of histamine secretion than does ergosterol.

TABLE 2

Blood pressure changes compared to changes in the rate of gastric secretion after intravenous injection of 5 per cent calcium lactate or normal saline or 1.2 per cent sodium lactate

TIME	MARCH 29				APRIL 2*		APRIL 5		APRIL 9†	
	1st injection		2nd injection							
	B.P.	Vol.	B.P.	Vol.	B.P.	Vol.	B.P.	Vol.	B.P.	Vol.
Before 13 mgm. Ca	112	5.8	104	12.0	58	7.2	100	6.4	96	3.4
2–3 min. after 13 mgm. Ca	150		200		72+		122+		136	
15 min. after 13 mgm. Ca.	110	4.4	100	5.3			112	2.3	98	3.6
30 min. after 13 mgm. Ca	110	5.3			54	2.6	104	1.4	96	2.0
45 min. after 13 mgm. Ca.			94	1.6			106	1.1	84	2.7
60 min. after 13 mgm. Ca.					56	7.3			86	2.8
75 min. after 13 mgm. Ca.			76	13.3						
Before 0.9 per cent NaCl	100	5.5	126	9.6			)	6.2		
2–3 min. after 0.9 per cent NaCl	112		112							
15 min. after 0.9 per cent NaCl	112	5.4	112	10.0				6.3		
30 min. after 0.9 per cent NaCl								8.1		
45 min. after 0.9 per cent NaCl	112	5.5	104	10.9			96	7.7		
Before Na lactate									126	6.9
2–3 min. after Na lactate									134	
15–30 min. after Na lactate.									134	6.3
45–60 min. after Na lactate.									112	5.0
75–90 min. after Na lactate.									112	6.8

* Splanchnics cut.
† 6.5 mgm. Ca.

In solving the question of whether or not parathormone or ergosterol inhibits more or less specifically the nervous or vagal type of secretion, these experiments can be of little value; however, they should be valuable in giving information on the effect *per se* of hypercalcemia. It appears that this has no specific action—both types of secretion being inhibited.

The relation between inhibition and hypercalcemia can be seen by com-

paring the reduction in secretion caused by the full amount (13 mgm. per kgm.) of calcium with that caused by fractions of the dose. In many experiments, too, the 2nd and 3rd doses of calcium, injected when the volume of secretion had returned to normal after the 1st, caused as great,

TABLE 3

Changes in blood flow through the stomach and systemic blood pressure changes after injection of calcium (13 mgm./kgm.)

TIME	BLOOD FLOW	BLOOD PRESSURE	REMARKS
	cc./min.	*mm.*	
Before calcium	12.80	136	
3 min. after calcium	30.00	164	
20 min. after calcium	12.50	136	
30 min. after calcium	12.50	126	
45 min. after calcium			Time of maximum inhibition
60 min. after calcium	9.12	110	
90 min. after calcium	9.08	86	Inhibition ended
98 min. after calcium	6.60	74	B.P. lowered artificially
100 min. after calcium	6.80	86	
110 min. after calcium	12.00	98	B.P. raised by calcium injection

TABLE 4

Blood calcium changes after the 1st injection of calcium (13 mgm./kgm.) compared with observations of Smith and Sternberger

TIME	BLOOD CALCIUM		NUMBER OF SAMPLES	BLOOD CALCIUM FIGURES FROM EXPERIMENT OF SMITH AND STERNBERGER (11.3 MGM. Ca/KGM. INJECTED)
	Average	Variations		
	mgm. per cent	*mgm. per cent*		*mgm. per cent*
Before calcium	10.1	9.3–11.0	8	9.9
4 min. after calcium				15.4
15 min. after calcium	16.0		1	13.4
30 min. after calcium	13.9	13.4–14.4	3	12.5
45 min. after calcium	12.7	11.9–13.5	4	
60 min. after calcium	13.1		1	
90 min. after calcium	12.8		1	11.0
105 min. after calcium	12.7		1	
120 min. after calcium	11.9	11.8–12.0	2	
135 min. after calcium	11.0		1	
5 hrs. after calcium				9.5

* Marks the end of the period of inhibition of gastric secretory rate.

or even greater reduction in the rate of secretion than the initial one, even though they were smaller. This I associated with the fact that the blood calcium was restored to normal more slowly than the volume of secretion (tables 1 and 4); thus the 2nd and 3rd injections were made into blood con-

taining calcium 2 to 3 mgm. above the normal level, which would appear to be the threshold. In apparent contradiction to the inter-relationship of these two factors, we have the events of the 15-minute period immediately following the injection when the blood calcium is at its maximum, but still no inhibition occurs. This could be perhaps partially explained by the sharp transitory rise in blood pressure which takes place in this period, accompanied by the increase in blood flow through the stomach; these circulatory changes, by increasing the head of pressure, may be obscuring a direct action of calcium on the gastric glands immediately after the injection.

A point which may prove to be of some significance but for which, at present, I have no explanation to offer, is the difference in the degree of inhibition between the group in which the secretion from the whole stomach was being measured and that in which the pylorus was excluded; in the latter the average reduction was less and the period of inhibition shorter.

CONCLUSIONS

In acute experiments on dogs the injection of calcium lactate or calcium chloride inhibits both the nervous and the chemical type of secretion.

The inhibitory agent appears to be the calcium radicle, since equivalent amounts of lactate as the sodium salt failed to have the inhibitory effect of either of the calcium salts. The degree of inhibition depends to some extent on the degree of hypercalcemia.

The hypercalcemic effect, under the conditions of these experiments, is not specific for nervous or chemical responses of the gastric glands, and in this differs from the action of parathormone and ergosterol in the chronic experiments of Babkin and his co-workers.

The mechanism for the inhibition has not been found. Osmotic pressure changes in the blood were not caused by the solutions and circulatory factors, i.e., general blood pressure and blood flow through the stomach could not be correlated with the changes in secretion rate caused by the calcium.

I am indebted to Prof. B. P. Babkin, who suggested, and maintained an unfailing interest in, this problem.

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THE RELATION OF CALCIUM CONTENT TO ACIDITY AND BUFFER VALUE OF GASTRIC SECRETIONS

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Variations in the calcium content of gastric juice were observed in an investigation (1) in which the inhibitory effect of hypercalcemia on gastric secretion was being studied. The variations bore an inverse relationship to the acidity of the secretion. Such a relationship has been observed recently, independently of us, by Kirsner and Bryant (2). Apart from this, isolated observations of calcium changes made in the course of studies of the composition of gastric juice have been reported. Some of these lend support to our results, without having received particular attention from their authors.

A systematic study of the relations between the calcium content and the acidity of gastric juice in dogs under various conditions of secretion are reported in this paper.

LITERATURE. Rosemann (3) reported the calcium content of gastric juice in the dog after sham-feeding to be 0.22 to 0.07 mgm. per 100 cc. Gamble and McIver (4) in a study of the acid-base composition of juice from Heidenhain pouches in cats during fasting and at intervals after food reported 4.1 mgm. in the fasting state, 10.6 mgm. 2 to 4 hours after food, and 5.7 mgm. 8 to 12 hours after food. Rudd (5) in two papers dealing with the composition of gastric juice in man found 4.1 to 8.6 mgm. per cent. This author observed that calcium was present in minimal amounts some time after the injection of histamine. Bonne, Hartz *et al.* (6) found 3.4 to 4.4 mgm. per cent in the secretions obtained with alcohol or caffeine tests in different racial types. Austin and Matthews (7) found an average of 6.08 mgm. per cent in 11 normal dogs, and of 6.65 mgm. per cent in 12 made hypercalcemic with parathyroid extract. The experiments of Kirsner and Bryant (2) were performed on 70 patients—5 with the Ewald test meal, 65 with histamine—and on 5 dogs, using either fasting or fundic pouch secretions. Values for the human cases ranged from 0.85 to 7.00 mgm. per cent and in general the higher the value the higher the pH found. It was also noted that the calcium was higher in fasting or Ewald test-meal secretions than in those after histamine, but that patients with achlorhydria were exceptions to low values with his-

tamine. In the fasting secretion of a pregnant dog the calcium ranged from 11.07 to 14.8 mgm. per cent, and in the histamine secretion was 11.07. The values for fundic pouch secretions of 4 dogs were much lower, i.e., 1.10 to 3.30.

The calcium variations to be described were found in the dog under acute conditions. These variations were accompanied by changes in other factors; the acidity bore an inverse, and the buffer value a direct, relationship.

Methods. Details of the experimental procedure have been described in the previous paper (1). Each 15-minute sample of secretion, after having been measured, was filtered through ashless filter paper and placed

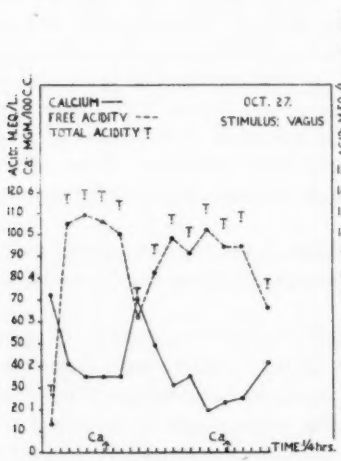

Fig. 1

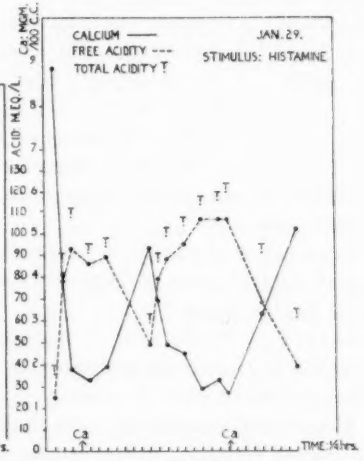

Fig. 2

Figs. 1 and 2. Reciprocal relationship between acidity and calcium concentration of gastric juice.

in the cold until the next morning, when it was analysed. Total and free acidity were determined with Töpfer's and phenolphthalein indicators; total chloride by the modified Volhard-Harvey method (8); pepsin by Mett's method as modified by Nierenstein and Schiff (9); and calcium by treating the filtered secretion as an acid extract of ashed material as in the Tisdall and Kramer method for calcium in urine and other biological materials (10). Values obtained by this method were the same as those found after ashing the secretion. In pyloric secretions where the viscosity was very high the material was ashed.

Results. The most obvious result of these experiments was the reciprocal relationship between the calcium concentration and the acidity of the secretion. Evidence that a high acidity is accompanied by a low

calcium and *vice versa* was seen under three different conditions: *a*. Following the intravenous injection of calcium salts. Figures 1 and 2 are typical examples of a total of 29 injections into 17 animals. The relationship held for secretions of both the histamine and the vagus type. *b*. During the control period before the calcium injection (table 1). It was a practical experience that the initial levels of acidity in any animal gave a general indication of the inital amount of calcium to be expected—whether high or low. *c*. The third type of evidence was free of certain criticisms which might be made of the first two—viz., of *a*, that the conditions of an artificial hypercalcemia are not normal, and of *b*, that individual differences from one experiment to another may lead to erroneous conclusions. In the third type vagal and histamine stimuli were given alternately in the same experiment (fig. 3). To obviate the criticism that depletion of the calcium stores might have caused the lower values in the second or histamine period which occurred in the first experiments, the order of the

TABLE 1

Comparison of initial levels of acidity and calcium concentration

ACIDITY	CALCIUM CONCENTRATION		NUMBER OF EXPERIMENTS
	Average	Variations	
m.eq./l.	*mgm./100 cc.*	*mgm./100 cc.*	
25– 75	6.67	3.73–8.70	4
76–100	4.05	1.47–6.40	7
101–125	2.97	0.55–4.89	9
126–150	1.91	0.46–4.19	4

stimuli was reversed but the vagal stimulus following the histamine had little if any effect. The other course open was to try to use the vagus in a 3rd period of stimulation after the hypothetical exhaustion of the calcium stores. This proved to be successful in spite of the fact that the final period of vagal stimulation followed a histamine period. This was attributed to the unusually small amount of histamine necessary to start and maintain a good volume of secretion, after the initial vagal period, compared to that necessary to yield a corresponding volume when the histamine was used alone. Thus the amount of histamine was not great enough under the former conditions to abolish the effect of the second vagal stimulus which followed it. In this experiment (fig. 3) we switch from a vagal secretion of low acidity and high calcium to that characteristic of histamine with high acidity and low calcium. In the final period a reversal to the characteristic vagal type is occurring, which shows that the low calcium values in the histamine period were not due to exhaustion of the calcium. This experiment has been repeated 5 times in cats with the same results.

Two exceptions to the reciprocal relationship between calcium and acidity may be of importance. In a number of cases in which free acid was absent in the initial samples the calcium was not at its maximum height, as might be expected, but rose to a higher level on the appearance of free acid. In the other exceptional case, the unusual combination of high levels of acidity and high "buffer values" (cf. below) in some samples were accompanied by very high calcium values.

The possibility that the changes in calcium concentration were simply reflections of the volume changes, with which it is generally accepted the acidity varies, is not supported by the finding that in the 21 cases in which

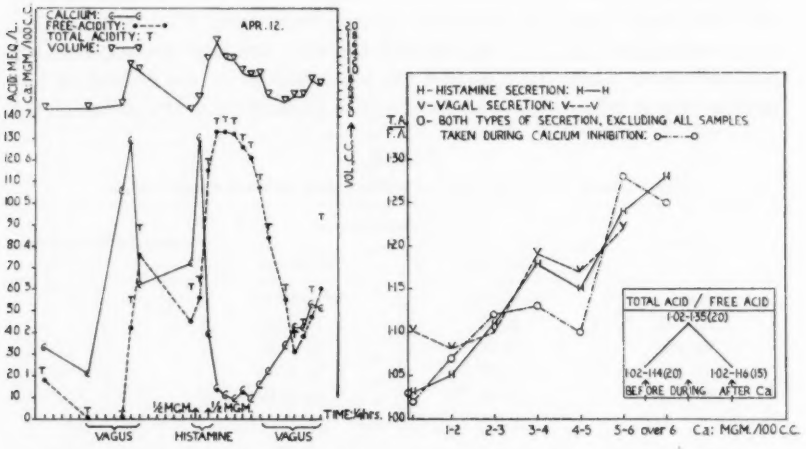

Fig. 3. Left. Changes in calcium and acidity caused by alternate vagal and histamine stimuli.

Fig. 4. Right. Relationship between calcium and ratio $\frac{\text{Total acid}}{\text{Free acid}}$.

Inset curve: Ratios before, during and after calcium—showing range of values and (in brackets) number of cases.

the data were complete for changes after calcium injection—i.e., those in which the initial volume was restored after the calcium effect had worn off—the acidity was lower and the calcium concentration higher than the control levels when the volume had already been restored after the period of maximum inhibition. Also when vagal and histamine stimuli were used alternately, in spite of parallel volumes in some periods, the characteristic differences in acidity and calcium for the two types of secretion were seen (fig. 3).

The variations in calcium output in these experiments lend support to the belief that the volume changes are not entirely responsible for the

changes in calcium concentration. When the decrease in output below the initial level occurring during the period of maximum inhibition of secretion was balanced against the subsequent increase in output, the balance was positive by amounts varying from +2 per cent to +204 per cent in 14 cases; in 7, however, the loss which occurred was not made up—the deficit amounting to from −8 per cent to −57 per cent. The calcium output increased when the vagal stimulus replaced the histamine.

TABLE 2

Total acidity/Free acidity ratios and corresponding calcium values in vagal and histamine types of secretion

	VAGAL SECRETION		HISTAMINE SECRETION	
	Ratio	Calcium	Ratio	Calcium
	m.eq./l.	*mgm./100 cc.*	*m.eq./l.*	*mgm./100 cc.*
March 1 (Dog)				
Average	1.09	4.65	1.02	0.43
Variations	1.06–1.23	1.83–13.20	1.02–1.03	0.00–1.07
Number of cases	6	6	7	7
March 8 (Cat)				
Average	1.08		1.03	
Variations	1.07–1.10		1.03–1.05	
Number of cases	5		7	
April 12 (Dog)				
Average	1.20	3.83	1.03	0.79
Variations	1.08–1.55	1.68–6.47	1.03–1.04	0.45–1.98
Number of cases	13	13	6	6

TABLE 3

SECRETION FROM	CALCIUM		TOTAL NUMBER OF CASES	SPREAD OF CALCIUM VALUES		
	Average	Variations		0-5 mgm. per 100 cc.	5.1-10 mgm. per 100 cc.	Over 10 mgm. per 100 cc.
	mgm./100 cc.	*mgm./100 cc.*				
Pyloric part	9.8	1.9–23.1	30	1	20	9
Fundus + corpus	1.7	0 – 4.6	25	25	0	0

An increase in the ratio Total acidity/Free acidity accompanied the increase in calcium concentration, whether this was due to injection of calcium or to replacement of histamine by vagal stimulus. This relationship also held when the control values of different experiments were compared. (Fig. 4 and table 2.)

The calcium concentration in pyloric secretion (always very viscous) was higher than in that collected over the same period from the rest of the stomach. In table 3 some comparative figures are listed.

Discussion. Independently of volume changes a high calcium and low acidity appear in gastric secretion after the intravenous injection of calcium and during vagal stimulation in contrast to the lower calcium and higher acidity occurring before the calcium injection and during histamine stimulation. This behaviour is most convincing when the changes are caused in a physiological way by stimulating alternately the cells responding to histamine and to the vagus. The association between these two factors is at present obscure. If the source of the calcium is some component of vagal secretion, as appears probable, the effect of acid coming in contact with this in the early stages of secretion and its disappearance finally from the acid secretion are problems at present being considered.

The increase in the ratio Total acidity/Free acidity constantly appears with an increase in calcium. If, as Rudd (5) concludes from his experiments on the effect of mucoprotein on the titration of HCl to the phenolphthalein end-point, when the titration is carried on from the Töpfer's end-point to that of phenolphthalein, the mucoprotein dissolves in the added alkali and the difference in the two readings is due mainly to the combination of NaOH and mucoprotein—then the differences in the ratios in our experiments are a measure of the amount of mucoprotein or other buffer present. The ratio then is an index of the buffer value. There appears to be an association between this factor and the amount of calcium occurring in the samples of secretion.

SUMMARY

Variations in the calcium concentration of gastric secretions of both the nervous and the chemical type obtained under acute conditions from dogs bear a reciprocal relationship to the acidity of the secretions.

This reciprocal relationship was found when the calcium and acidity changes were either spontaneous or induced; viz. 1, when comparing the initial levels of calcium and acidity in 24 animals; 2, in single experiments when vagal and histamine stimuli were used alternately to produce their characteristic secretions of low or high acidity respectively; 3, when the factors were changed by intravenous injection of calcium salts.

The relationship is not dependent on volume changes. The output of calcium increased after the period of inhibition caused by calcium injection and during vagal stimulation.

The ratio Total acidity/Free acidity which is interpreted here as an index of the buffer value varies directly with the calcium changes brought about in the ways mentioned above.

I am greatly indebted to Prof. B. P. Babkin, in whose Department this work was performed, for his interest in the problem.

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THE DISTRIBUTION OF INJECTED RADIOACTIVE POTASSIUM IN RATS

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The predominantly intracellular distribution of potassium is well established, but the mechanism by which this intracellular concentration is maintained is not well understood. With the development of an artificially radioactive isotope of potassium K^{42}, a new method of attack upon the problems associated with the metabolism of this element has become available. We have employed this new technique to measure the magnitude and rate of total uptake of injected potassium by various types of tissues, thus gaining an insight into the relative permeabilities of different cells to potassium.

Two previous studies on the distribution of K^{42} after injection have been published. Greenberg et al. (1938) and Joseph, Cohn and Greenberg (1939) used albino rats and determined the rate of uptake of the labelled atoms per gram of tissue for muscles, liver, kidney and blood (but not plasma) after intraperitoneal or oral administration. Hahn, Hevesy and Rebbe (1939) have described the distribution of K^{42} in a single rabbit 24 hours after subcutaneous injection and also in the plasma, muscle and bone of a few frogs. In both of these investigations, however, adequate interpretation of the results is impossible because of lack of analyses for the total potassium of the tissues under consideration although Hahn et al. have made use of certain assumed potassium values in the interpretation of their results.

In our work we have determined total potassium as well as radioactivity of tissue samples taken at varying times after the injection of K^{42}. This is necessary in order that the results may be interpreted properly, for the radioactivity of any tissue depends first of all upon the amount of potassium which it contains in a form available for exchange with its radioactive isotope. It has become evident that injected potassium passes into all tissues of the body, but at varying rates which are high in liver, heart, kidney and lung, intermediate in muscle and skin, and low in testes, erythrocytes and brain. Approximate equilibrium is secured about six hours after injection, but red cells and brain are not in complete equilibrium

even after 18 hours. In general our results are in agreement with data published by Joseph, Cohn and Greenberg but are in conflict with the findings of Hahn, Hevesy and Rebbe that very little exchange of potassium between plasma and tissue cells occurs.

METHODS. Albino rats were used as the experimental animals, usually without previous fasting or other preliminary treatment.

The activated potassium was prepared by deuteron bombardment of samples of chemically pure potassium chloride weighing 100 to 150 mgm. In order to free the sample from possible contamination with radioactive sodium, the activated potassium salt was dissolved in a minimal volume of water and precipitated as the perchlorate in a Gooch filter crucible by the addition of saturated ammonium perchlorate solution. The potassium perchlorate precipitate was washed and converted to the chloride by heating in the presence of a trace of manganese dioxide. After being weighed the potassium chloride was dissolved in the crucible, filtered by suction and made up to a volume of 10 ml. The loss of weight of the crucible was determined and was assumed to represent the potassium chloride in the solution.

The radioactive solution, containing usually about 1 per cent potassium chloride, was injected by one of several routes. After varying intervals of time, the animals were sacrificed by cutting the throat, and the blood was collected by holding the wound over a beaker. Weighed samples of tissues (0.5 to 1.0 gram) were dissolved in concentrated nitric acid in tared weighing bottles or graduated centrifuge tubes. From the volume of the digest (either read directly on the graduate or calculated from the weight of the digest and its measured specific gravity) suitable aliquots were taken both for determination of the radioactivity by a Geiger-Müller counter as modified by Bale, Haven and LeFevre (1939) and for total potassium content by the Shohl and Bennett method with minor modifications (Fenn and Cobb, 1936). A suitable dilution of the injected K^{42} solution was used as a standard in measuring the radioactivity of the tissue samples. After deduction of the background count (determined with 3 ml. of distilled water around the ionizing chamber in place of 3 ml. of digest) the K^{42} counts were plotted against time on "semi-log" paper. Linear interpolations between points on this graph served for the determination of the simultaneous standard count for each sample count. The sample counts were then expressed as a percentage of the simultaneous K^{42} standard count. This procedure corrects for the decay of the radioactive isotope as well as for any changes in the efficiency of the counter. Standard count and background counts were made approximately every two hours while the samples were being counted. The background counted was usually about 2 per minute and the standard and samples about 10 to 100 per minute. Most solutions were counted for 15 minutes, or longer if the count was very low.

The following calculations have been made from the data:

$$\text{Activity} = \frac{\text{counts found per kgm. wet weight of sample}}{\text{total counts injected per kgm. body weight}}$$

$$\text{Potassium activity} = \frac{\text{activity} \times 100}{\text{millimols of potassium per kgm. of wet tissue}}$$

$$\text{Relative potassium activity} = \frac{\text{potassium activity of tissue}}{\text{potassium activity of plasma}}$$

$$\text{Exchanged potassium in mM per kgm. body weight} = \frac{100}{\text{potassium activity of plasma}}$$

When mixing is complete the exchanged potassium is the same as the total potassium in the body. The activity has a value of 1.0 when the injected potassium is evenly distributed throughout all the body weight. The potassium activity has been multiplied by 100 merely for convenience. It may be noted, however, that this is not far from the number of millimols of K per kilogram body weight of an average rat. If the correct figure were known in each case and were used in place of 100 then the potassium activity so calculated would have a value of 1.0 when the injected potassium was evenly distributed throughout all the body potassium. The relative potassium activity ordinarily has a value of 1.0 when exchange between a given tissue and the plasma is on the average complete. There is no way of knowing of course whether all parts of the tissue sample were equal in their potassium activity. Some parts of the sample may have been higher and others lower than the plasma.

Results. The results of a single complete experiment are given in detail in table 1. The table is self-explanatory. It will be seen that only 85 per cent of the injected counts were recovered. The discrepancy is presumably due to various small losses during the experiment. The loss in the excretion was 9.2 per cent which includes both urine samples and probably feces. The figure 1.30 for the potassium activity of the urine is based on analysis of the second urine sample and presumably represents the average potassium activity of the plasma during the last 13 hour collection period. The K activity of the plasma at the end of that time was somewhat lower (1.045).

In table 2 are listed most of the actual figures obtained in our experiments on rats. Values are given for the activity (act) and the total potassium content (K) in m.eq. per kgm. wet weight of the various tissues. From these two values, the potassium activity may be calculated. The experiments are arranged in order of duration and are grouped according to the method of administering the radioactive isotope. Dosage, calculated total potassium, and average potassium contents for the various tissues are also included in table 2.

In eight experiments including that shown in table 1 an attempt was made to recover from the rat all the counts injected. This involved

TABLE 1

Protocol of typical experiment—18 hour rat, no. 26

4/16/40 3:30 p.m.—192 gram male rat from stock, injected subcutaneously with 4 ml. 1.47 per cent radioactive KCl solution and placed in metabolism cage.

4/17/40 8:30 p.m.—Urine collected, sample A. 9:30 a.m.—Urine collected, sample B. Rat killed by cutting throat, blood collected with oxalate by holding wound over beaker, tissue samples dissected, weighed and dissolved in nitric acid.

TISSUE	WEIGHT OF SAMPLE	COUNTS PER MIN. CORRECTED	ACTIVITY	POTASSIUM	POTASSIUM ACTIVITY	RELATIVE POTASSIUM ACTIVITY	COUNTS IN WHOLE SAMPLE, PER CENT OF TOTAL
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	grams			*mM/kgm.*			
Plasma	1.65	834	0.00802	7.66	1.045	1.00	0.07
Liver	5.4	11,150	0.107	102.6	1.045	1.00	3.01
Kidney	1.325	9,660	0.093	79.1	1.17	1.12	0.64
Heart	0.616	9,900	0.095	83.8	1.13	1.08	0.31
Gastro-int.	12.4	7,500	0.072	62.8	1.15	1.10	4.65
M. gastrocnemius	1.100	13,750	0.132	116.2	1.14	1.09	0.76
Red cells	2.2	8,380	0.081	97.2	0.83	0.79	0.92
Brain	1.215	5,160	0.050	106.0	0.47	0.45	0.31
Testes	2.018	8,170	0.084	94.0	0.89	0.85	0.88
Skin	45.9	3,880	0.037	52.8	0.70	0.67	8.90
Diaphragm	0.443	11,015	0.105	86.9	1.21	1.16	0.24
Lung	1.104	9,350	0.089	83.8	1.06	1.01	.52
Residue	111.0	9,850	0.095	82.4	1.15	1.10	54.67
Excreta					1.30	1.24	9.20
Total	186.37						85.08

The weights given in column 2 represent the whole organ or tissue except in the case of muscle where only one gastrocnemius was used. Some blood was lost in cutting the throat. Column 3 is expressed in terms of the counts per min. given by the simultaneous standard solution. The standard used was the injected solution diluted 500 times. Column 3 is the counts per 5 min. in a 3 ml. sample of the nitric acid digest $\times$ (total vol. of digest $\div$ wt. of sample) $\div$ simultaneous standard count $\times$ 1000. The standard used was the injected solution diluted 500 times. Column 4 is column 3 $\div$ 10.41×10^6, the number of counts injected per kgm. body wt. The number of counts injected was 4 ml. $\times$ 500 (dilution) $\times$ 1000 (for convenience) = 2×10^6. Column 5 is calculated from the analysis of the nitric acid digest. Column 6 is col. 4 $\div$ col. 5 $\times$ 100. Column 7 is col. 6 $\div$ 1.045. Column 8 is col. 3 $\times$ col. 2 $\div$ $2 \times 10^6 \times 100$.

The exchanged K $= \frac{100}{1.045} = 96$ millimols K per kgm. Adding up all the K found in the samples and the residue gives a total of 74.3 millimols per kgm. The discrepancy is partly due to loss of K in the excreta which was not accounted for.

sampling all the tissues as usual and in addition dissolving everything remaining after the dissection (carcass) and analyzing it as a whole for

TABLE 2

Activity (Act.) and potassium content (K) of various tissues

RAT NO.	ROUTE	TIME	WEIGHT	CALC. TOTAL K	DOSE OF K	PLASMA		LIVER		KIDNEY		HEART		GI		MUSCLE		ERYTHRO-CYTES		BRAIN		SKIN		TESTES		DIA-PHRAGM		LUNG	
						Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K	Act.	K
		hours	*gm.*	*m.eq./kgm.*	*m.eq./kgm.*																								
1	IP	0.5	151	59	2.8	0.151	9.1	3.04	92	1.57	40	1.99	91	2.49	79	0.69	116	0.12	86	0.05	103	0.48	48						
2	IP	0.5	153	55	(2.3)	0.153	8.6	2.83	89	0.87	76	2.04	80	0.81	62	0.69	112	0.18	103	0.06	96	0.26	38					0.84	63
3	IP	1.0	237	86	(2.2)	0.133	11.8	3.27	109	1.44	80	1.61	93	1.99	60	0.62	121					0.26	44						
4	IP	1.0	202	66	(1.7)	0.141	9.6	5.96	96	1.58	89	1.98	95			0.66	94	0.18	78	0.14	92								
5	IP	1.0	316	46	1.1	0.104	4.9	4.16	(91)							0.23	118	0.28	99	0.08	(102)								
6	IP	1.0	215	87	3.2	0.070	6.4	0.45	95	0.99	86	1.25	89			0.13	114	0.08	83	0.03	113							0.60	86
7	IP	1.0	240	63	(2.2)	0.151	9.8									0.02	129	0.15	90										
8	IP	1.0	318	66	2.0	0.178	11.7	3.26	91							1.35	112	0.18	88	0.11	108								
9	IP	1.6	230		1.0	0.184		4.07	91	1.93	75	2.07	86			1.66	117												
10	IP	2.0	242		1.0	0.145		3.37	(91)			1.96	(84)			1.10	(113)												
11	IP	2.0	200	37	2.7	0.183	5.6	7.50	99	2.32	53	3.54	89			1.88	117	0.62	97	0.20	105			0.61	95	5.64	86	2.66	71
12	IP	3.0	252	83	2.8	0.088	7.6	2.32	101	1.41	82	1.16	81	1.46	59	1.44	76	0.40	86			0.50	(43)					0.91	(81)
13	IP	3.0	312	46	0.9	0.187	9.7	2.93	(91)			2.18	82			1.96	114					0.56	36						
14	IP	5.0	291	105	0.7	0.090	9.6	1.76	92	1.28	70	1.39	83	1.51	88	0.96	120	0.50	96	0.28	110			0.54	93	1.76	100	1.41	83
15	IP	5.0	289		0.7	0.114		1.23	75	0.86	66	1.17	87	1.67	102	1.47	120	0.34	76	0.25	106					1.58	100	1.08	78
16	IP	6.5	184	64	2.8	0.166	11.0	1.75	109	0.74	64	1.51	(84)	1.31	83	2.32	122	0.55	97	0.28	109								
17	IP	10.0	195	98	(3.6)	0.110	11.0	1.01	90	1.07	73	0.88	75	1.13	74	1.36	123	0.55	101	0.33	94	0.49	39	0.58	76				
18	ST	1.0	208	83	2.8	0.146	12.6	3.50	107	1.62	83	1.68	82	3.25	80	0.55	104	0.15	84	0.08	110	0.33	46	0.12	96			1.54	70
19	ST	2.0	193	67	3.3	0.116	8.2	2.63	101	1.76	79	2.05	87	2.72	80	1.08	117	0.21	100	0.15	98	0.56	45	0.21	96				
20	SC	1.0	246	69	0.8	0.173	12.0	1.28	84	1.65	79	1.89	91	0.86	65	0.66	113	0.17	95							1.45	92	1.13	80
21	SC	1.0	268	71	0.3	0.12	8.4	1.29	102	2.12	(75)	2.20	(84)													1.45	110	1.04	98
22	SC	1.0	230	38	2.3	0.200	8.4	2.04	94	1.56	86	1.72	79	1.24	90	0.78	115	0.16	96							1.06	90	0.85	74
23	SC	1.0	256	66	0.3	0.102	6.8	1.66	92	1.51	(75)	1.48	(84)	1.15	69	0.67	112	0.15	91							1.48	99		
24	SC	2.0	190	86	2.7	0.095	8.4	1.60	88	1.62	81	1.71	87	1.48	91	1.00	119	0.23	95	0.13	102	1.52	48	0.25	90				
25	SC	6.0	134	75	4.0	0.094	7.4	1.30	91	1.04	78	1.23	80	0.28	48	1.44	111	0.42	78	0.30	99	0.63	43					0.98	85
26	SC	18.0	192	91	4.1	0.080	7.7	1.08	103	0.92	79	0.96	84	0.73	63	1.32	116	0.81	97	0.50	106	0.36	53	0.85	94	1.06	87	0.90	84
Average				70			8.85		95		75.6		84.7		70.4		112.0		93.4		102.4		43.3		92.1		91		80.9

The amount of the injected K has been deducted from the calculated total K. Potassium contents (K) are expressed as milliequivalents per kilogram wet weight. Rats 4 to 7, 11 and 15 were anesthetized with urethane and no. 8 was treated with metrazol. No. 5 had one sciatic nerve cut. No. 6 had both denervated and one of them stimulated. No. 7 had one sciatic cut and stimulated; no. 4 had one intact sciatic stimulated. IP = intraperitoneal; SC = subcutaneous; ST = stomach tube. The majority of the muscle figures are averages of determinations on 2 different muscles. Figures in ( ) represent values assumed for purposes of calculation.

radioactivity and for potassium. The results of these eight experiments are summarized in table 3. Column 4 gives the total potassium per kilogram of whole rat. This figure was obtained from analyses of the carcass after adding in the weights and potassium contents of all the samples. The variation in different animals is surprisingly large and appears to be well beyond the experimental error. The average value is 66.4 m.eq. per kgm. or 0.26 per cent potassium. This is close to the value of 0.25 per cent reported by Heppel and Schmidt (1938), and also close to the average value of 69.8 m.eq. per kgm. for the total exchanged potassium as calculated in table 2. In most of the experiments the rat was kept in a metabolism cage so that the excreta could be collected and analyzed for radioactivity. The contents of the bladder at the time of

TABLE 3

Total balance and distribution of injected radioactive potassium

RAT NO.	TIME	ROUTE	TOTAL K	COUNTS RECOVERED	CARCASS COUNT	DISTRIBUTION IN PER CENT OF COUNTS INJECTED								
						Excreta	Liver	Skin	G.I.	Muscle	Blood	Heart	Brain	Kidney
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	hours		*mM/kgm.*	*per cent*										
1	0.5	IP	62.0	78	69	10.4	11.4	8.7	16.5	28.4	0.6	0.79	0.05	0.76
2	0.5	IP	74.0	85	137	1.9	14.1	4.7	6.2	30.6	0.7	0.87	0.03	0.61
18	1.0	ST	60.9	61	57		14.7	6.0	13.2	22.4	0.8	0.62	0.05	1.36
13	3.0	IP		118	55	2.9	7.9	10.1		78.8	0.7	0.23		
25	6.0	SC	74.0	92	243	10.1	5.1	11.5	1.2	57.4	1.3	0.45	0.22	0.77
16	6.5	IP	56.6	89	512	9.0	6.7		4.6	84.8	1.1	0.45	0.09	0.28
17	10.0	IP	62.8	75	119	6.8	3.2	8.8	4.3	54.6	1.7	0.30	0.26	0.95
26	18.0	SC	74.3	85	481	9.2	3.0	8.9	4.7	52.6	2.7	0.31	0.31	0.64

IP = intraperitoneal; SC = subcutaneous; ST = stomach tube.

death as well as the contents of the gastro-intestinal tract were also included. This figure in per cent of the total counts injected per kilogram body weight is given in column 7 (excreta). There is no regularity in these figures because they include material not absorbed as well as material excreted both in feces and urine. In rat 18 the KCl was given by stomach tube and 44 per cent of the amount was found in the G-I tract at death. This figure was so large that (in this experiment) the distribution in the various tissues has been expressed in per cent of the amount absorbed.

The percentage of the total counts injected which were recovered either in the rat or in the excreta is given in column 5. On the average only 86 per cent was recovered. The large variations from this figure are probably due to errors in the counting because the variations are especially large

The amount of the injected K ... 7, 11 and 15 were anesthetized with urethane and no. 8 was treated with metrazol. No. 5 had one sciatic nerve cut. No. 6 had both denervated and one of them stimulated. No. 7 had one sciatic cut and stimulated; no. 4 had one intact sciatic stimulated. IP = intraperitoneal; SC = subcutaneous; ST = stomach tube. The majority of the muscle figures are averages of determinations on 2 different muscles. Figures in ( ) represent values assumed for purposes of calculation.

where the number of counts for a 5-minute period found in the carcass sample was especially low as shown in column 6. We are unable to account satisfactorily for the low average recovery of the injected counts. The amounts injected were measured with as much accuracy as a 5 ml. syringe permits.

In calculating the distribution of the injected counts in the various tissues listed in table 3, it was assumed that the skin represented 18 per cent and the muscles 40 per cent of the body weight. In the last experiment the skin was actually removed in toto and ashed and analyzed separately. In this case it amounted to 24 per cent of the body weight, but a similar procedure in experiment 2 gave a value of 17.9 per cent. In the other tissues actual weights of the whole liver, the whole G-I tract, etc., were obtained. On account of the assumption that 40 per cent of the body weight is muscle and the further assumption that all the muscles contained the same amount of K^{42} per gram as the gastrocnemius or rectus femoris muscles, which were the ones usually analyzed, the fraction of the injected dose allotted to muscle must be regarded only as a rough estimate. In some cases it is obviously too large for it amounts to nearly as much as all the counts recovered. For this reason the sum of columns 7 to 15 cannot be expected to add up to the value in column 5 for total counts recovered. It is evident, however, that muscles account for a larger fraction of the total counts than any other tissue.

In figure 1 the plasma potassium activities have been plotted against time. The scatter of the points is very large because each point represents a different rat. Intraperitoneal, subcutaneous and stomach tube experiments have all been included but no consistent differences are apparent. The plasma potassium activity rises immediately after the administration of the potassium but the active potassium is disposed of so quickly in the tissues by exchange or transport that the activity reached in the plasma is never much greater than the final equilibrium value. The graph shows also the scale for the amount of exchanged potassium which can be calculated by dividing the plasma value into 100. The graph indicates a maximum exchanged potassium of 95 m.eq. per liter whereas the total in the body is not over 70. This discrepancy is attributed to two different factors: 1, excretion of 5 to 10 per cent of the injected counts; 2, higher potassium activity in some of the tissues than in plasma due to incomplete mixing. Furthermore, if the activity were calculated in relation to the total counts found in the whole body instead of the total injected, the plasma K activity would have had nearly the expected value of about 1.4.

If the plasma activity be plotted instead of the plasma K activity the resulting curve is very similar in shape to that shown in figure 1. No significant differences between intraperitoneal and subcutaneous injections

are apparent and the final equilibrium value of the activity which is reached is about 0.1 with an average early peak value of 0.15. In one rat at 1 hour a maximum value of 0.2 was observed.

All these figures for activity and potassium activity of plasma refer, of course, to arterial blood and it is impossible to estimate how high the portal blood or the mixed venous blood may have been. The portal vein plasma must have been at least as high as the highest values for the K activity observed in the liver.

The similarity between the curves for plasma activity and plasma potassium activity when plotted against time indicates that there was little change in the concentration of potassium in the plasma even immedi-

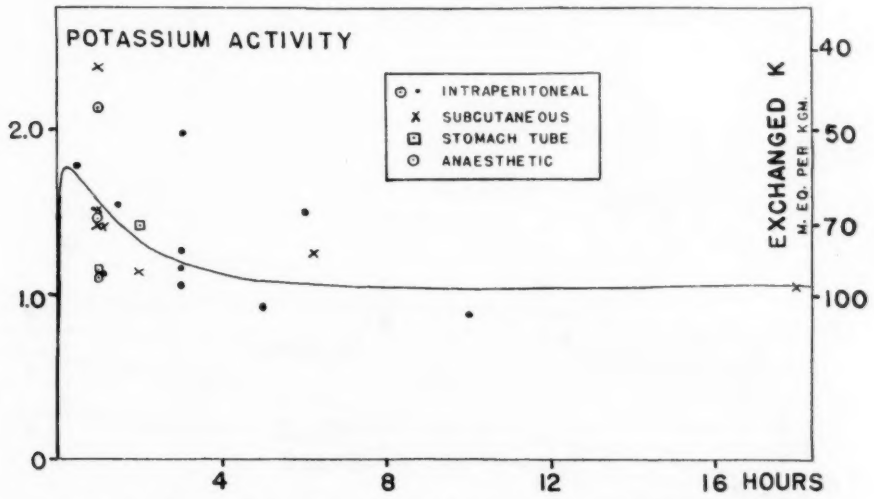

Fig. 1. Potassium activity of plasma and calculated exchanged potassium for various methods of injection.

ately after injection. Our data do not include figures for plasma K previous to injection but the rats killed within 1 hour after injection gave values only about 2 m.eq. per kgm. larger than the rats killed many hours later.

The figures presented for a few experiments in table 3 show that the injected potassium is found in continuously increasing amounts in muscles, brain and blood cells while the amount in the liver at least appears to pass through an early maximum and then to decrease. In general, a mobile fraction amounting to about 15 per cent of the injected radioactive K is lodged first in the viscera and then gradually moves over into the muscles and skin as mixing becomes complete. This same result appears from the

curves of figure 2 in which the relative potassium activity of liver, muscle, brain, and erythrocytes is plotted against time after injection. (This is the same as the potassium activity of the tissues corrected to a plasma potassium activity of 1.0.) The muscle curve shows a gradual rise until the relative potassium activity is equal to or slightly greater than the plasma value, whereas the liver curve rises quickly to values much greater than 1 and then slowly falls. When all curves have reached the plasma value the relative potassium activity is the same throughout the body and

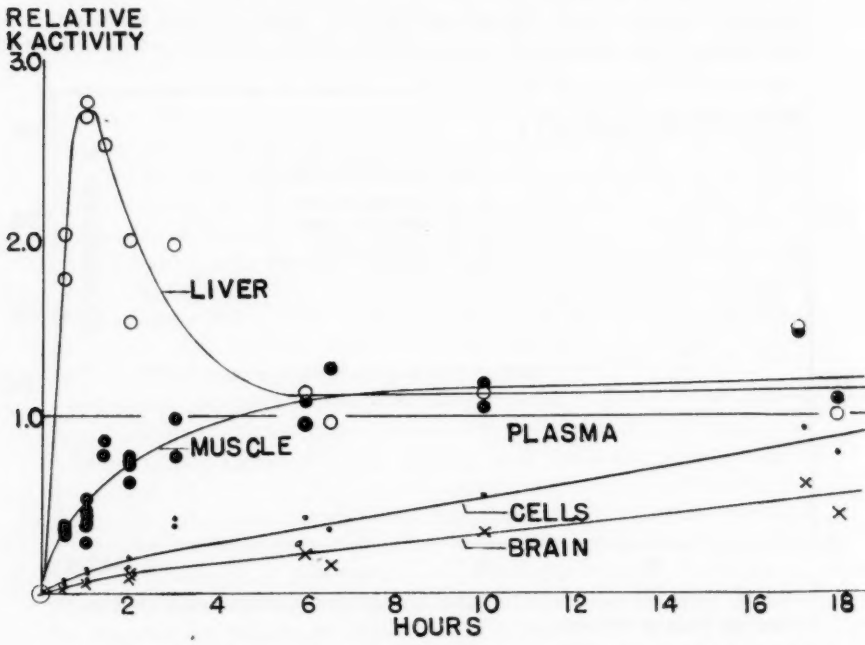

Fig. 2. Relative potassium activity of liver (only intraperitoneal administration plotted during first 4 hours), muscle, erythrocytes (cells), and brain. Value of 1 indicates complete equilibrium.

mixing may be said to be complete. We consider it likely that the muscle curve does go slightly above unity and then returns slowly to that value. In any event mixing is nearly complete in 4 to 6 hours so far as muscle and liver are concerned. Brain and erythrocytes rise even more slowly than muscle. The meaning of the liver curve will be discussed more completely later. It is sufficient to emphasize here that 1 hour after injection the liver may contain twice as many counts per mol of potassium as does the plasma. This indicates either a high degree of permeability to potassium

or a large mass movement of highly radioactive potassium from the plasma to the liver or both.

The liver and the muscle represent two categories into one of which all the tissues of the body appear to fall. In figure 3, for example, are plotted similar curves for the heart and the skin, the former behaving like the liver

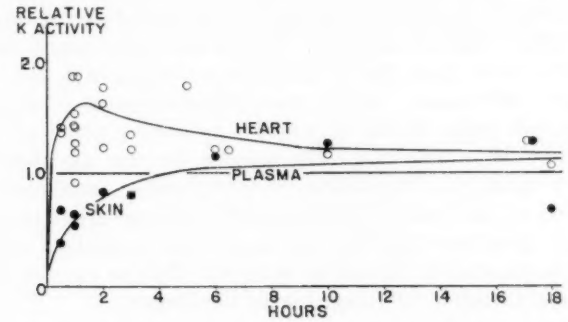

Fig. 3. Relative potassium activity of heart and skin. Value of 1 indicates complete equilibrium between tissue and plasma.

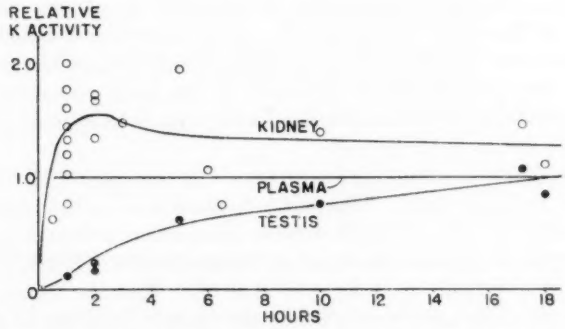

Fig. 4. Relative potassium activity of kidney and testis. Value of 1 indicates complete equilibrium between tissue and plasma.

and the latter like the muscle. Likewise in figure 4 the kidney behaves like liver while testis resembles muscle.

It was thought possible that the high activities observed in the liver after intraperitoneal injection might be due to contamination from unabsorbed potassium in the peritoneum during dissection. In some experiments, therefore, we washed out the peritoneum and analyzed the washings separately without finding more than a few per cent of the injected dose present after 30 or more minutes and only 15 per cent after

10 minutes. After intraperitoneal injection, the absorbed material would pass first through the liver from the portal circulation so that this tissue would be exposed to a plasma with a higher potassium activity than any other tissue in the body. To avoid this situation a number of animals were injected subcutaneously and, in two cases, by stomach tube. The results of subcutaneous injection differ from those with intraperitoneal injection only in the case of liver, gastro-intestinal tract, diaphragm, and possibly lung. Administration by stomach tube yields results not differing significantly from those found after intraperitoneal injection. Figure 5 shows these differences. In all cases, intraperitoneal or stomach tube administration leads to higher relative potassium activities, especially for the short time intervals. The reasons for this are that the liver, alimentary canal, and diaphragm enjoy favorable anatomical positions with regard to the venous and lymphatic drainage from the peritoneal cavity and therefore would be in contact with a solution richer in K^{42} than would tissues less favorably situated. In the case of the lung it seems probable that the difference is not significant.

Discussion. Radioactive potassium in the plasma will disappear into the tissues: *a*, by a possible mass movement or transport of potassium in solution, that is, an ingestion of an intracellular potassium solution by cells, or *b*, by exchange of radioactive potassium in the plasma with normal potassium in the cells. There will also be an excretion of labelled potassium in the urine, but this will be relatively small because the radioactive potassium is so quickly mixed with most of the body potassium and the absolute amount of potassium excreted while this is happening is so small compared to the total in the body.

The participation of both these processes of transport and exchange is clearly shown by the data. Thus the potassium level in the plasma is not much increased in spite of the absorption of an amount of potassium equal on the average to 15 times the total plasma potassium. Since the absorption is nearly complete in 15 minutes it is clear that the transport of an equal amount of potassium into the tissues is also complete in this time. According to previous data, injected potassium appears to distribute itself in the body in proportion to the body water. (Winkler and Smith, 1938; Wilde, 1938; Fenn, 1939.) The expected increase in plasma potassium is therefore only about 2 mM per liter which is not far from the observed increase. Without any transport into cells and with all the injected potassium confined to the extracellular spaces the increased concentration in the plasma would have been 2 to 3 times as large.

Clear evidence that K^{42} must also disappear from the plasma by exchange for K^{39} from the tissues is found in the fact that the potassium activity rises quickly to a maximum *and then decreases*. A transport of mixed plasma potassium into the tissues (without exchange) could decrease the

activity of the plasma but could not alter the potassium activity which would increase to a maximum during absorption of K^{42} and would not decrease unless potassium of lower potassium activity entered either from ingested food or by exchange from the tissues, or unless the tissues took up K^{42} in preference to K^{39}.

While both transport and exchange contribute to the distribution of radioactive K in the body it is noteworthy that by the process of transport

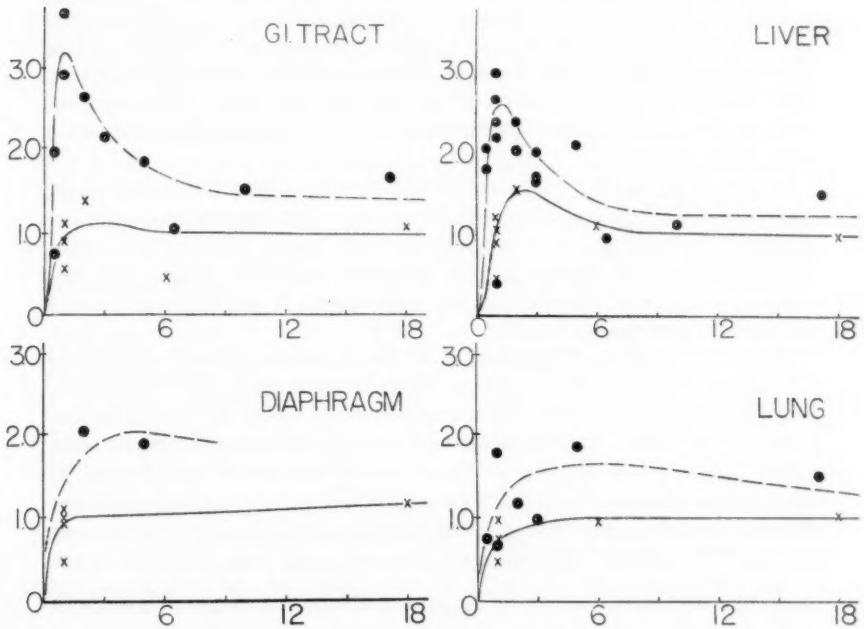

Fig. 5. Ordinates—relative potassium activity of liver, gastro-intestinal tract, diaphragm, and lung after intraperitoneal and stomach tube (● —— ● ——) and after subcutaneous (× —— × ——) administration. Value of 1 indicates complete equilibrium between tissue and plasma. Abscissae—time in hours.

the K^{42} would probably be distributed evenly in all the body water whereas by the process of exchange it would be distributed evenly in all the body potassium. Since the latter is the observed result it is evident that complete exchange does eventually occur even though the excess load of K injected is distributed according to a different rule.

We may now consider what changes in tissue activity are to be expected simply as a result of ionic exchange of K^{42} for K^{39}. Since the potassium activity of the plasma passes through a maximum and then falls it is to

be expected that the tissues will tend to do likewise. Those tissues which are most permeable to potassium will also show a considerable peak in potassium activity, but this peak will lag somewhat behind the plasma peak whereas tissues which are relatively impermeable to potassium will show scarcely any peak, and this will occur very late. Since the rate of change of the activity in any tissue will be zero when the potassium activity inside is just equal to that outside it is evident that the peaks in potassium activity shown by the various tissues should fall exactly on the curve for potassium activity in the plasma.[1] Unfortunately our present data are scarcely adequate to enable us to decide whether they conform to this theoretical condition or not, because there are too few experiments at very short durations, and the scatter of the points is too great. Moreover we have no data for the portal blood plasma which is necessary for comparison with liver.

On the other hand a transport of radioactive K into the tissues could also cause tissue potassium activities greater than simultaneous plasma values if the transport were greater into some tissues, such as liver, than into others such as muscles. The difference would be enhanced if the portal blood plasma entering the liver had a higher K activity than arterial plasma which feeds the muscles.

Evidence for greater transport of K into liver than into muscles was obtained in this laboratory (Fenn, 1939) by analyzing cat livers before and after injection of KCl. It was concluded, subject to certain limitations, that the liver, which accounts for only about 3 per cent of the body weight, was nevertheless able to absorb 7 to 15 per cent of the injected K. Two similar experiments have been performed on rats and the data are shown in table 4. The rats were etherized and samples of the liver were removed for analysis. Immediately after recovery from the ether 5 ml. of $\frac{m}{10}$ KCl was given to each rat by stomach tube. Two hours later both rats were killed, and samples of blood and liver were taken for analysis. The liver was thus analyzed before and after KCl for K, H_2O and blood content and the blood was analyzed for K. In interpreting the results it was assumed that the total weight of dry liver did not change. The wet weight of the liver before injection was calculated from that found after injection on this basis. The amount of blood in the liver was determined by mincing a small piece of liver in a measured volume of isotonic salt solution and counting the red cells. Red cell counts were also made in whole blood. These data permitted a correction of the total liver K for blood K. The results of the 2 experiments show that 7.6 and 6.4 per cent respectively of the potassium dose was located in the liver. Chloride

[1] A more mathematical application of this theory to red blood corpusles will be found in a paper by Dean, Noonan, Haege and Fenn (1940).

spaces were determined also in experiment 1 and found to be somewhat less after injection. A correction for extracellular K, therefore, would increase still further the calculated percentage of the dose found in the liver. These experiments, therefore, support the idea that the high potassium activities in the liver are due in large measure to transport or mass movement of K from a portal blood plasma of high potassium activity. The process of exchange may well be a much slower process, thus accounting for the ease with which the K activity of the plasma can fall below that in the liver, heart, and other viscera. It is even possible that exchange is no more rapid in liver than in muscle, all the difference between the tissues being accounted for by transport of material of high K activity from the

TABLE 4

Potassium in rat liver before and 2 hours after giving 5 cc. of m/10 KCl by stomach tube

	EXPT. I		EXPT. II	
	Before	After	Before	After
Weight of liver, wet*	8.17	8.38	10.05	9.96
K, m.eq. per kgm. wet	99.3	100.2	99.3	102.5
K, m.eq. per kgm. dry	320	331	324	331
Dry weight, per cent	31.03	30.26	30.61	30.97
Blood, per cent	5.15	2.99	3.7	1.65
K in blood, m.eq. per kgm.		53.0		57.8
Total K minus blood K, m.eq.	0.789	0.827	0.979	1.011
Excess K after injection, m.eq.		0.038		0.032
Excess in per cent of amount fed		7.6		6.4

* The weight before injection was calculated from the dry weight of the liver found after KCl. The body weights were 245 and 248 grams respectively in experiments 1 and 2. In experiment 1, the chloride space of the liver was 28.8 per cent before and 22.2 per cent after injection, the K contained in this space being 0.018 and 0.014 m.eq. respectively. The blood in the liver was lower after injection because the samples were taken after bleeding the animal to death.

portal blood plasma. In a preliminary abstract of this work (Noonan et al., 1940) we interpreted our results in this sense but further consideration shows that this view is not yet completely proved. The relative quantitative importance of exchange and transport in the results must still be measured.

SUMMARY

At different times after administration in various ways of radioactive potassium to albino rats the animals were killed and samples of plasma, red cells, and various tissues were analyzed both for total potassium and for radioactivity. The radioactive potassium was found to penetrate rapidly into most of the tissues of the body, relatively little being found

in the plasma. The rate of penetration is highest in liver, heart, kidney, lung, diaphragm, and gastro-intestinal tract, intermediate in muscle and skin, and low but not absent in testes, erythrocytes and brain.

In tissues where penetration is rapid the activity per mol. of total potassium present in the first 1 to 2 hours is 1.5 to 2.5 times as high as the simultaneous plasma value. The difference was especially great after intraperitoneal injection, but was still present after subcutaneous injection.

Since the increase in total K in the plasma is only a small fraction of the total K injected, elimination of K from the blood must have occurred by mass movement of K or by exchange with some other cation. The low value of the activity in the plasma on the other hand indicates a nearly complete mixing of radioactive K with all the normal K in the body. Both processes indicate widespread permeability to K.

After equilibrium is established the bulk of the radioactive potassium is found where the bulk of the total body potassium is, i.e., in the muscles, with skin and viscera next in importance.

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MEASUREMENT OF THE BLOOD FLOW OF THE LIVER

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The rate of flow of blood through the liver was measured in dogs by the thermostromuhr method. The measurements of blood flow included the flow in the hepatic artery, portal vein and thoracic inferior vena cava, the abdominal inferior vena cava having previously been ligated. The study was made in order to obtain data on the inflow of blood to the liver and outflow of blood from it as a basis for further studies on trained dogs under various physiologic conditions.

The flow of blood from the hepatic veins has been determined in several investigations by means of a mechanical stromuhr. The average value for anesthetized dogs has been reported to be 84 cc. per minute per 100 grams of liver by Burton-Opitz (1), and 64 to 144 cc. per minute per 100 grams of liver by Macleod and Pearce (2). In anesthetized cats Barcroft and Shore (3) found the flow to be 33.3 to 48 cc. per minute and Schmid (4) reported a value of 54 cc. per minute per 100 grams of liver. Blalock and Mason (5) collected and measured the blood flowing through the hepatic veins in dogs weighing from 11.5 to 18.8 kgm. under local anesthesia; they reported the flow to be 216 to 612 cc. per minute, averaging 387 cc. per minute (27 cc. per minute per kilogram of body weight or 82 cc. per minute per 100 grams of liver). Grab, Janssen and Rein (6, 7) measured the total hepatic blood flow in dogs after recovery from anesthesia with the thermostromuhr. The average flow was 65.3 cc. per minute per 100 grams of liver. The average flow through the thoracic inferior vena cava in five dogs ranged from 250 to 860 cc. per minute, while the flow through the abdominal inferior vena cava ranged from 60 to 340 cc. per minute. Soskin, Essex and two of us (Herrick and Mann) (8) used the thermostromuhr and reported the flow through the thoracic inferior vena cava of anesthetized dogs, when the abdominal inferior vena cava was ligated, to be from 40 to 160 cc. per minute per 100 grams of liver. Using the same method and preparation, Essex, Baldes and two of us (Herrick and Mann) (9) found the flow through the thoracic inferior vena cava in a trained dog weighing 7.8 kgm. to be 408 to 552 cc. per minute.

Average values for flow of blood in the hepatic artery of anesthetized dogs have been 143.4 cc. per minute (10), 15 to 55 cc. per minute (11),

and 30 to 65 cc. per minute in dogs after recovery from anesthesia (6). Similar values for blood flow in the portal vein of anesthetized dogs have been 268.2 cc. per minute (1), 80 to 200 cc. per minute (11), 100 to 330 cc. per minute (12), and 125 to 340 cc. per minute in dogs after recovery from anesthesia (6).

METHOD. Ligation of the inferior vena cava between the entrance of the hepatic veins and that of the right lumbo-adrenal vein had been performed on all the dogs several months previously. Usually this operation was done in two stages but in an occasional experiment evidence of sufficiently developed collateral veins permitted completion of the ligation to be delayed until the time of the experiment. The weight of the dogs varied from 11.5 to 25.5 kgm. and averaged 16.76 kgm. The flow of blood in the hepatic artery, portal vein and thoracic inferior vena cava was measured by the thermostromuhr method (13). Thermostromuhr units were placed on these vessels while the dogs were under anesthesia. The units, which ranged from 2 to 6 mm. in diameter, were calibrated in vitro on several occasions before and after an experiment and were found to be reliable within the limits of accuracy of the method. When blood flow was studied while the dogs were under anesthesia, pentobarbital sodium was used as the anesthetic agent. Ether anesthesia and aseptic technic were used for applying the units in those animals in which the blood flow was studied after recovery from anesthesia.

A midline or right rectus incision afforded access to the portal vein and hepatic artery. Care was taken to avoid enlarged collateral veins in the abdominal wall. The pancreaticoduodenal artery and vein were ligated in the critical experiments, the former at its origin from the hepatic artery and the latter at its junction with the portal vein. A thermostromuhr unit was placed on the hepatic artery proximal to the ligated pancreaticoduodenal artery in the critical experiments. This procedure was the most difficult and time consuming, great care being required in the freeing of the hepatic artery from its investment of splanchnic nerves. A few nerve fibers were unavoidably traumatized. The portal vein unit was applied caudal to the point of entry of the ligated pancreaticoduodenal vein when liver blood flow was measured during anesthesia. When flow was measured after recovery from anesthesia, the unit was placed on the portal vein above the pancreaticoduodenal vein.

The abdominal incision was then closed and artificial respiration under positive pressure was started. The right pleural cavity was opened by an incision in the sixth thoracic interspace, and the thoracic inferior vena cava exposed. Freeing of the middle portion of this vessel and application of a thermostromuhr unit were accomplished very quickly. The chest wound was then closed and artificial respiration discontinued except in a few of the experiments in which blood flow was measured during anesthesia.

The flow of blood was recorded on a moving photographic film. By this means it was possible to determine accurately the flow of blood in several vessels simultaneously and continuously. However, a few direct

TABLE 1

*Hepatic blood flow of a group of dogs during pentobarbital sodium anesthesia**

DOG	WEIGHT	NUMBER OF OBSERVATIONS	BLOOD FLOW IN CUBIC CENTIMETERS PER MINUTE			
			Hepatic artery	Portal vein	Inflow (hepatic artery plus portal vein)	Outflow (thoracic inferior vena cava)
	kgm.					
1	11.8	11	95	321	416	Not measured
2	15	1	(122)	(230)	(352)	(435)
		1	(88)	(200)	(288)	(292)
		5	55.4	248	303.4	252.4
		1	(75)	(145)	(220)	(267)
		5	86	151.8	237.8	269
3	18.8	1	(105)	(450)	(555)	(500)
		15	106	393	499	447
		1	(110)	(505)	(615)	(465)
		1	(99)	(420)	(519)	(565)
		8	102	345	447	455
		1	(105)	(365)	(470)	(520)
4	16.5	1	(163)	(218)	(381)	(390)
5	18.0	1	(112)	(365)	(477)	(460)
		7	51	335	386	351
		1	(53)	(435)	(488)	(465)
		9	44.6	407.4	452	478.7
		1	(47)	(420)	(467)	(370)
6	11.5	1	(61)	(203)	(264)	(344)
7	25.5	1	331	260	591	620
8	17.0	1	(440)	(130)	(570)	(590)
		11	478.5	100.2	578.7	557.4

* When a series of simultaneous observations were made, the table gives the number of such observations and the average value of the blood flow in each vessel during the short interval covered by the observations. The series of eleven observations on dog 8 were made at half minute intervals; at other times a series of observations represented simultaneous readings at one minute intervals. Single observations are shown enclosed in parentheses in the table in order to indicate that the observations were from three to five minutes apart. The observations on dogs 2 and 5 were made with the chest open and with positive pressure artificial respiration.

observations of flow in each vessel were always made and such observations were not made simultaneously.

RESULTS. During pentobarbital sodium anesthesia the usual blood flow ranged from 44.6 to 163 cc. per minute in the hepatic artery, and from 145 to 505 cc. per minute in the portal vein (table 1). Although the portal

vein flow was usually two to three times that of the hepatic artery, two dogs (dogs 7 and 8, table 1) had larger hepatic artery flows than portal vein flows. The blood flow in the thoracic inferior vena cava of the group of anesthetized dogs ranged from 252.4 to 620 cc. per minute (table 1). After recovery from ether anesthesia, the usual flow in the portal vein of a group of dogs ranged from 206.5 to 473.9 cc. per minute, and from 368 to 507.5 cc. per minute in the thoracic inferior vena cava (table 2). The portal vein flow and thoracic inferior vena cava flow were measured simultaneously in some experiments after recovery from anesthesia (dogs 9 and 11, table 2), and the hepatic artery flow was calculated by subtraction of portal flow from thoracic inferior vena cava flow. The values of hepatic artery blood flow thus obtained were 33.6 and 65 cc. per minute.

TABLE 2

*Blood flow in the portal vein and thoracic inferior vena cava in a group of dogs on the first or second day after recovery from ether anesthesia**

DOG	WEIGHT	DAY	FLOW IN CUBIC CENTIMETERS PER MINUTE		CALCULATED HEPATIC ARTERY FLOW
			Portal vein	Thoracic inferior vena cava	
	kgm.				
9	17.5	1	303	368	65.0
10	19.4	1	206.5		
		2	330		
11	22.0	2	473.9	507.5	33.6
12	21.4	1		505	

* The data represent typical values obtained on the first and second days after operation. Both the portal vein and thoracic inferior vena cava blood flow were measured in dogs 9 and 11. The calculated hepatic artery flow for these dogs represents the difference between the thoracic inferior vena cava flow and portal vein flow.

When the rate of flow of blood was measured in two or more vessels simultaneously by a photographic recording of the flow in these vessels, it was observed that the flow in each vessel was constant within certain limits. This has been illustrated by a typical experiment on one dog (dog 3, table 3) in which simultaneous measurements of hepatic artery, portal vein and thoracic inferior vena cava flow at one minute intervals show no wide fluctuations. However, subtraction of outflow from total inflow revealed that there were small differences at each interval, and that frequently the magnitude of these differences increased and decreased in cyclic fashion. When simultaneous measurements of inflow and outflow over a period of time were plotted graphically, cyclic changes in inflow and outflow, which were out of phase with each other, could be seen

(fig. 1). This suggests that the differences between inflow and outflow were caused by rhythmic storage and discharge of stored blood by the

TABLE 3

Dog 3: Pentobarbital sodium anesthesia. Blood flows in the hepatic artery, portal vein and thoracic inferior vena cava were recorded simultaneously at one minute intervals for a period of eight minutes

TIME	FLOW IN CUBIC CENTIMETERS PER MINUTE			
	Hepatic artery	Portal vein	Inflow (hepatic artery plus portal vein)	Outflow (thoracic inferior vena cava)
minutes				
1	102	340	442	440
2	99	355	454	440
3	102	355	457	420
4	99	345	444	465
5	105	355	460	440
6	95	340	435	450
7	107.5	330	437.5	465
8	107.5	340	447.5	520
Average.....	102	345	447	455

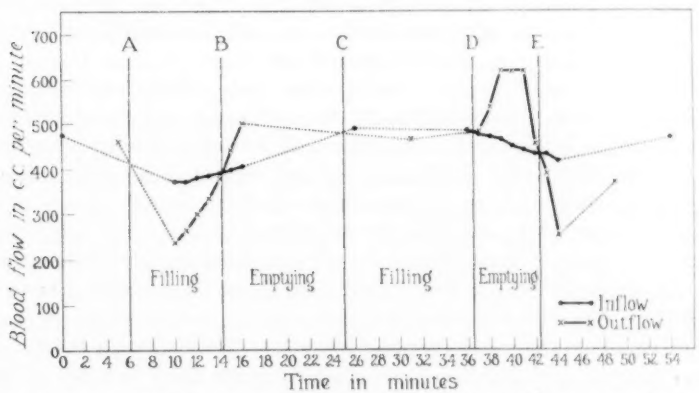

Fig. 1. Dog 5: pentobarbital sodium anesthesia. Liver inflow and outflow during an interval of about fifty minutes. Inflow of blood was computed by addition of values for simultaneous observations of flow in the hepatic artery and portal vein. The outflow of blood was measured in the thoracic inferior vena cava. Inflow exceeded outflow during the A-B and C-D intervals, and outflow exceeded inflow during the B-C and D-E intervals. Broken lines indicate intervals during which blood flow was not recorded photographically.

liver. In the presence of this phenomenon it is evident that inflow and outflow would only occasionally be equal in magnitude.

COMMENT. After each experiment was concluded examination of the abdominal inferior vena cava at the site of ligation revealed that the occlusion had been complete. There was no evidence that total inflow through the portal vein or the hepatic artery was not measured. Small veins in the central portion of the diaphragm entering the thoracic inferior vena cava above the liver were noted but they could not have contributed greatly to measurements of liver outflow. Occasionally these small veins were ligated.

Measurement of the blood flow of the liver by the technic used in this study has given values which are of approximately the same magnitude as values of previous studies on dogs. The flow of the hepatic artery during pentobarbital sodium anesthesia in six experiments ranged from 44.6 to 163 cc. per minute. The calculated flow of the hepatic artery after recovery from ether anesthesia ranged from 33.6 to 65 cc. per minute. These values may be compared with values of 143.4 cc. per minute (Burton-Opitz, 10), 30 to 65 cc. per minute (Grab and co-workers, 6, 7), and 15 to 55 cc. per minute (Schwiegk, 11). The flow of the portal vein during pentobarbital sodium anesthesia in six experiments ranged from 145 to 505 cc. per minute. The usual portal vein flow after recovery from anesthesia ranged from 206.5 to 473.9 cc. per minute. Others have obtained values of 268.2 cc. per minute (Burton-Opitz, 1), 125 to 340 cc. per minute (Grab and co-workers, 6, 7), 80 to 200 cc. per minute (Schwiegk), and 100 to 330 cc. per minute (Katz and Rodbard, 12).

In two experiments (dogs 7 and 8, table 1) it will be noted that the relationships of the blood flow in the hepatic artery and the portal vein are reversed in respect to the values for flow in these vessels of other dogs of the study. Although the data do not explain the cause of the reversed relationship, they support the hypothesis that the hepatic artery under certain conditions may compensate for a lowered supply of portal vein blood to the liver. Burton-Opitz (1, 10) estimated the hepatic artery to carry about 30 per cent and the portal vein about 70 per cent of the liver blood flow. He (14, 15, 16) expressed the opinion, however, that there was a free reciprocal interchange between these two flows and that the hepatic artery flow compensated for low portal flow, the liver radicles of both vessels possessing motor activity. Macleod and Pearce observed that occlusion of the hepatic artery decreased liver blood flow about 30 per cent, of the portal vein about 60 per cent. However, they expressed the opinion that this relationship was variable and controlled by vasomotor activity, feeble in the case of the radicles of the portal vein in the liver. Grab, Janssen and Rein estimated the flow in the hepatic artery to be from 12 to 22 per cent of the total blood flow of the liver, while Schwiegk estimated it to be from 20 to 25 per cent, and Blalock and Mason estimated it to be from 12.6 to 24.5 per cent. Schwiegk found that warming of the

dog caused an appreciable increase of hepatic artery flow and decrease of portal vein flow. Soskin and co-workers (8) have stated that the share of blood entering the liver carried by the portal vein or hepatic artery may vary as much as from 10 to 90 per cent in either vessel.

Calculation of inflow of blood to the liver, by addition of hepatic artery and portal vein blood flow values, should give a result which closely approximates the measured outflow of blood through the thoracic inferior vena cava. In general, the outflow and inflow values were found to be in close agreement. However, differences in such values occurred and these were analyzed. If differences between inflow and outflow were due entirely to inaccuracy of the method of measurement of flow, one would expect random variations in the magnitude of the differences in a series of inflow and outflow observations. On the other hand, a consistent trend in magnitude might indicate a storage of blood or discharge of stored blood by the liver. An actual comparison of consecutive pairs of inflow and outflow observations disclosed the fact that differences usually had a certain relationship. This relationship indicated alternate storage and discharge of blood by the liver. A typical experiment illustrating such data has been presented in table 3 where an initial phase of filling of the liver appears to be followed by a phase of emptying. Similar data covering a longer interval have been presented graphically in figure 1. During such conditions it is obvious that the outflow of blood through the thoracic inferior vena cava would only at certain times be of the same magnitude as the combined portal vein and hepatic artery flow.

From this study it appears to us that the rate of blood flow of the liver is subject to many physiologic variations, such as rhythmic filling of the hepatic sinusoids. For this reason a correlation of liver blood flow with the size of the liver or animal is not always apparent and, indeed, should not be expected. Continuous observation over a period, as the thermostromuhr method permits, discloses wide physiologic variations. We have made similar observations using this method in studies of blood flow in other viscera. A single observation represents only the rate of blood flow under the conditions of the moment at which the observation was made. Because of its dynamic nature we believe that it is fallacious to represent liver blood flow as a static flow, in terms of the average flow for an animal, or in terms of rate of flow for a unit of liver or body weight.

The data obtained in this study merit one further consideration. While measurements of blood flow in the hepatic artery, portal vein and thoracic inferior vena cava by the thermostromuhr method show variations in the flow of these vessels, such variations do not incriminate the method. In general, simultaneous observation of flow in all these vessels discloses that wide fluctuations of flow in one vessel are reflected in compensatory changes in the flow of the other vessels. Smaller fluctuations in flow appear to be

concerned with rhythmic filling of the liver with blood. It is our belief that these consistently observed phenomena lend support to the reliability of this method for study of hepatic blood flow.

SUMMARY

The rate of flow of blood in the hepatic artery, portal vein and thoracic inferior vena cava was measured in a group of dogs by the thermostromuhr method. The abdominal inferior vena cava had previously been ligated. During pentobarbital sodium anesthesia the usual flow ranged from 44.6 to 163 cc. per minute in the hepatic artery, from 145 to 505 cc. per minute in the portal vein, and from 252.4 to 620 cc. per minute in the thoracic inferior vena cava. When total inflow and outflow of blood from the liver were measured, the values obtained were in close agreement.

After recovery from ether anesthesia the usual flow in the portal vein of a group of dogs ranged from 206.5 to 473.9 cc. per minute, and from 368 to 507.5 cc. per minute in the thoracic inferior vena cava. The calculated hepatic artery flow ranged from 33.6 to 65 cc. per minute.

Low blood flow in the portal vein in two anesthetized dogs was accompanied by high blood flow in the hepatic artery.

Simultaneous observations of inflow and outflow indicate that under certain conditions the liver may alternately store blood and discharge stored blood.

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THE FORMATION OF HYDROXYTYRAMINE BY EXTRACTS OF RENAL CORTEX AND BY PERFUSED KIDNEYS

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In 1939, Holtz and co-workers (1) showed that the anaerobic incubation of renal cortical extracts with l-dopa (l-dihydroxyphenylalanine)[1] resulted in the formation of a pressor substance which was identified as hydroxytyramine. According to these investigators the formation of this substance from dopa was caused by a decarboxylase and took place only under oxygen lack. Under aerobic conditions, however, hydroxytyramine was quickly oxidized by an amino-oxidase to dioxyphenylacetaldehyde, a depressor substance. The fact that an oxygen-free atmosphere was necessary for the formation of hydroxytyramine suggested the possibility that a similar reaction takes place in kidneys in situ, made ischemic by Goldblatt's method (2). Therefore, experiments were performed to investigate the production of hydroxytyramine from dopa in isolated cat's kidneys perfused with a reduced blood flow. These results were compared with those obtained by the incubation of dopa and extracts of renal cortex under anaerobic conditions.

METHODS. The method of Holtz (1) was employed in the preparation of hydroxytyramine by incubation of dopa and renal extracts. Guinea-pig kidneys were used, as they are rich in dopa-decarboxylase and thus are more active than those of other species in forming the pressor substance. Twelve grams of cortex from three animals were ground in a mortar with quartz sand and extracted for five minutes with 125 cc. of M/20 disodium phosphate buffer (pH 7.4). The mixture was then centrifuged for fifteen minutes and the supernatant fluid mixed in an Ehrlenmeyer flask with 125 mgm. of dopa dissolved in 10 cc. of distilled water. After adding a few cubic centimeters of toluene, nitrogen was bubbled through the solution for five minutes. Then the flask was sealed and the solution was incubated at 39°C. for eight hours. At the end of this time, 7.5 cc. of 50 per cent trichloracetic acid were added and the solution was filtered with suction.

[1] Hoffman-La Roche.

The protein-free filtrate was extracted three times with ether to remove excess trichloracetic acid. Finally, the solution was distilled to dryness in vacuo at 60°C., the residue dissolved in 10 cc. of water, neutralized with NaOH, and used for injection.

Cats anesthetized with nembutal (0.6 cc. per kgm. body weight, intraperitoneally) were used in all experiments. In the perfusion experiments, an apparatus was used which permitted parallel perfusion of several organs under pulsatile pressure in a recirculating system. The perfusate consisted of heparinized blood. The apparatus will be described in detail in a further communication.

EXPERIMENTS. *Experiments with kidney extracts.* In six experiments, 1 cc. of the protein-free solution made from 12 grams of renal cortex was injected into a test cat. In one case the blood pressure rose from 140 to 230 mm. Hg, the rise lasting for three and one-half minutes (fig. 1). Similar results were obtained in the other five experiments. The injection of 6 mgm. of dopa dissolved in 1 cc. of Ringer's solution had no effect. It will be seen from figure 2 that, after injection of cocaine hydrochloride (6 mgm. per kgm. body weight, intravenously), the pressor effect of the kidney extract was enhanced. Since Holtz (1) has shown that cocaine potentiates the action of hydroxytyramine, it is possible that the rise in blood pressure resulting from the injection of the kidney extract was caused by hydroxytyramine.

In three experiments the influence of oxygen upon the production of hydroxytyramine from dopa by renal cortex was investigated. The disodium phosphate buffer extract of 16.2 grams of guinea pig cortex was divided into three samples of equal volume. The first sample was incubated under nitrogen with 65 mgm. of dopa dissolved in 20 cc. of water; the second, with the same amount of dopa under oxygen; and the third, without dopa under nitrogen. The samples were left in the incubator for eight hours at 38°C. After deproteinizing, distilling to dryness, redissolving and neutralizing the solutions, 1 cc. of each portion was injected into the same animal and the blood pressure recorded. The injection of the extract incubated under nitrogen caused a rise in blood pressure of 140 mm. Hg, lasting for approximately three minutes; that of the extract incubated under O_2, of 75 mm. Hg. One cubic centimeter of the third portion produced only a slight rise. A second injection of the same sample, however, caused a fall in blood pressure.

From the results obtained with the solution incubated under oxygen it was apparent that under aerobic conditions the production of hydroxytyramine was diminished but not abolished. Since it has been claimed by Holtz and co-workers (1) that in the presence of renal extract and oxygen an enzymatic breakdown of hydroxytyramine occurs, it was considered possible that the discrepant result mentioned above was caused by an

overproduction of hydroxytyramine due to the presence of an excess of dopa. In this case not all of the amine could be oxidized to the aldehyde.

To investigate this problem 82 cc. of phosphate buffer extract made from 13.6 grams of renal cortex were divided into two equal parts. To each sample 35 mgm. of dopa were added. The first solution was kept under nitrogen; the other under oxygen. After incubation for eight hours the proteins were removed, the extracts distilled to dryness, and the residues were finally dissolved in 6 cc. of water in each case and then neutralized. In three experiments the injection of 2 cc. of the portion derived from the aerobic kidney extract caused no change in blood pressure, whereas an equal amount of the anaerobic extract had a pressor action. These experiments confirmed the observation of Holtz that a quick breakdown of hydroxytyramine takes place in the presence of renal cortex and oxygen, provided that the amount of dopa does not exceed the capacity of the amino-oxidase in the renal tissue.

Experiments on perfused organs. In order to ascertain whether the whole kidney has the capacity, possessed by renal cortical extracts, to transform dopa into hydroxytyramine, eight experiments were performed on the isolated organ. The kidney was excised according to the method of Steggerda, Essex and Mann (3). The perfusate consisted of 100 cc. of heparinized blood. The ventilated lung was included in the perfusion circuit to remove the constrictor substances normally present in shed blood (4). After constant blood flow through the kidney had been established, the rate of flow through that organ was reduced by partial occlusion of the inflow tube leading to the renal artery. This was accomplished by manipulation of the stopcock in that part of the perfusion apparatus. The pressure head in front of the occlusion was kept at 120/100 mm. Hg.

After forty-five minutes of perfusion 15 cc. of the perfusate were removed to serve as a control, and 200 mgm. of dopa, dissolved in 30 cc. of Ringer's solution, were added to the perfusion fluid. Perfusion then continued for an additional period of two hours. After that time the perfusate, as well as the control previously removed, were centrifuged for fifteen minutes and the plasma injected into a cat.

In one experiment the blood flow through the kidney was reduced from approximately 15 to 5 cc. per minute. Five cubic centimeters of plasma, obtained by centrifuging the kidney perfusate, were injected into the animal. Following the injection, the blood pressure rose from 100 to 220 mm. Hg, the rise lasting for four minutes (fig. 3). After the injection of cocaine hydrochloride (6 mgm. per kgm. body weight, intravenously), the injection of 5 cc. of the same plasma caused a rise of 125 mm. Hg, lasting for eight and one-half minutes. The control plasma had no effect.

In a second experiment, the rate of flow through the kidney was reduced from 15 to 8 cc. of blood per minute. The injection of 5 cc. of perfused

plasma raised the blood pressure from 100 to 190; after injection of cocaine, an equivalent amount of plasma raised the pressure from 140 to

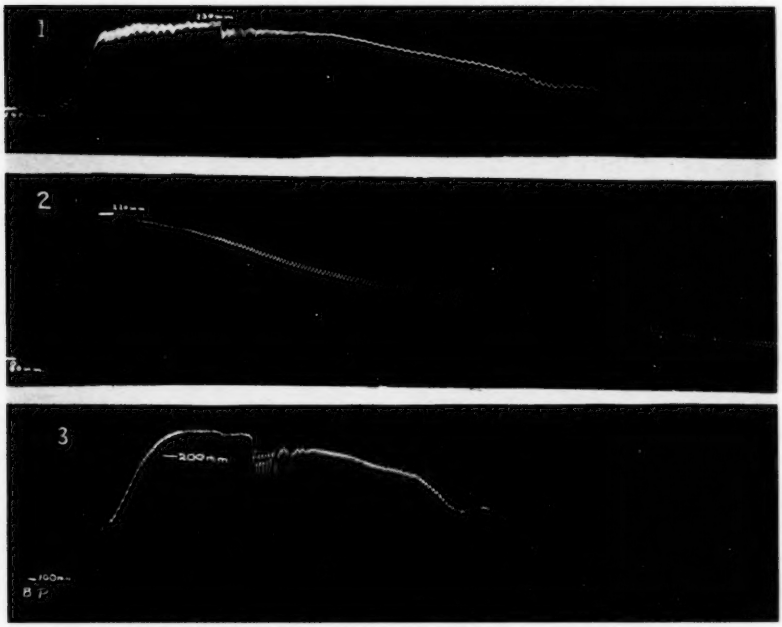

Fig. 1. Shows the effect of the injection of 1 cc. of the protein-free solution made from renal cortical extract anaerobically incubated with dopa. The blood pressure rises from 140 to 230 mm. Hg, the rise lasting three and one-half minutes. (July 9, 1940. Cat ♂, 3.2 kgm., nembutal anesthesia.)

Fig. 2. Shows the effect of the injection of 1 cc. of the same preparation after the injection of cocaine hydrochloride. The potentiating effect of the drug on the height and duration of the rise is noticeable. The blood pressure rises from 80 mm. to 220 mm. Hg, the effect lasting for four and one-half minutes. (Same animal as used in fig. 1.)

Fig. 3. Shows the action of 5 cc. of plasma obtained from kidney perfusate. The blood flow through the perfused kidney was reduced from 15 to 5 cc. per minute. Dopa had been added to the perfusate at the beginning of the perfusion. The blood pressure rises from 100 to 220 mm. Hg, the effect lasting four minutes. (August 20, 1940. Cat ♂, 3.4 kgm., nembutal anesthesia.)

250 mm. Hg. In this case, as in the preceding one, the injection of cocaine prolonged the duration of the rise in pressure.

In a third experiment the renal blood flow was lowered from 15 to 10 cc. per minute. Injection of 5.5 cc. of plasma, obtained by centrifuging the perfusion fluid, evoked only a slight rise in the blood pressure of the cat.

However, its duration was potentiated by cocaine, as in previous experiments.

It can be concluded that the perfused kidney has the capacity which is inherent in extracts of renal cortex to transform dopa into a pressor substance. The amount of this pressor substance produced depends on the blood flow through the kidney. The identity of the pressor substance produced by perfusion of the kidney with dopa dissolved in blood, and that resulting from the action of renal cortical extracts on dopa, is indicated by the fact that in each case oxygen lack is a necessary condition and that the action of the pressor substance produced by each of these two methods is potentiated by cocaine.

Since Holtz claimed (5) that the dopa-decarboxylase, which is necessary for the production of hydroxytyramine from dopa, is found in the liver and in the gut of the guinea pig, experiments were undertaken to determine whether the pressor amine could be produced from dopa by perfusion of the liver or gut of the cat. The liver was perfused through the portal vein at a pressure head of 25/15, the gut at 100/80 mm. Hg. In each case the lung was included in the perfusion circuit.

Perfusion of the liver took place for two hours. The perfusate consisted of 100 cc. of heparinized blood. After constant blood flow through the liver had been established, the rate of venous outflow from the organ was reduced from 60 to 10 cc. per minute. After thirty minutes of perfusion 15 cc. of blood were removed as control, and immediately afterwards 200 mgm. of dopa dissolved in 30 cc. of Ringer's solution were added to the perfusate. After completion of the perfusion 6 cc. of the perfused plasma were injected into a cat and the blood pressure was recorded. No rise was noticeable. The control plasma also failed to evoke a response.

The gut was perfused with 110 cc. of blood for one hour and thirty minutes through the superior mesenteric artery and the rate of blood flow reduced from 20 to 5 cc. per minute. After thirty minutes of perfusion 15 cc. of blood were drawn from the perfusate to serve as a control, and 220 mgm. of dopa were added to the perfusion fluid. After two hours the perfusate was removed and centrifuged. Five and one-half cubic centimeters of perfused plasma, as well as an equal amount of the control serum, injected into a cat, had no effect on the blood pressure.

In three cases it could be shown that blood alone, when incubated with dopa, does not form any of the pressor amine. This was demonstrated in experiments in which 40 cc. of heparinized blood were incubated at 38° with 100 mgm. of dopa under nitrogen for twenty-three hours. After that period, the blood was centrifuged and 5 cc. of the plasma injected. In two experiments a slight rise in the blood pressure was noticeable. However, the injection of cocaine hydrochloride abolished this effect. This indicates that the rise was not due to hydroxytyramine but to some

other pressor substance present in the blood (6). It was evident, therefore, that the perfused liver, the perfused gut and incubated blood could not transform dopa into hydroxytyramine. Consequently, of the organs studied, the kidney was the only one to possess this faculty.

Discussion. Experiments performed on anoxic kidney extracts indicate that under conditions of oxygen-lack, dopa (dihydroxyphenylalanine) is converted into a substance which raises the blood pressure of the cat. Cocaine enhances the rise as well as the duration of the response. It is the latter, however, which is mainly potentiated. It seems justified to conclude that the substance described in these experiments is identical with hydroxytyramine. This assumption is supported by the facts that: 1, Holtz' technique is followed as far as the experiments on renal extracts are concerned; 2, the pressor substance originates only in the presence of dopa and the absence of oxygen; and finally 3, its effect is enhanced by cocaine.

TABLE 1

REDUCTION IN THE RATE OF VENOUS OUTFLOW FROM THE KIDNEY	RISE IN BLOOD PRESSURE
cc./min.	*mm. Hg*
12	120
8	104
7	90
5	30
No reduction	No rise

In investigating the influence of oxygen upon the formation of hydroxytyramine it was found that the yield of pressor substance was decreased under aerobic conditions. This may be explained by an amino-oxidase which, according to Holtz (1), is found in the renal cortex and in the presence of oxygen transforms hydroxytyramine into dihydroxyphenylacetaldehyde, a depressor substance. The fact that some pressor amine was obtained in one case in which the reaction was carried out in the presence of oxygen can be explained by the fact that an excess of dopa was present, and the amino-oxidase was unable to oxidize all the hydroxytyramine which had been formed. This is confirmed by the finding that when smaller concentrations of dopa were used, no trace of a pressor substance could be detected.

The experiments on the perfused kidney indicate that the intact organ transforms dopa into a pressor substance which is secreted into the perfusion fluid. This was demonstrated in experiments in which the perfused plasma raised the blood pressure of the cat. Elevations as high as 120 mm. Hg lasting for eight minutes were obtained. It can be assumed

(1) Copied from a reprint of the article.

that hydroxytyramine is the active agent produced by the perfused kidney, since: 1, cocaine enhances its action, and 2, the presence of dopa and anaerobic conditions are necessary factors in its production.

Reduction of the renal blood flow is essential in the formation of the amine. This is demonstrated in experiments which showed that the reduction of the blood flow through the kidney paralleled the amount of hydroxytyramine present in the perfusate.

Since the pressor amine could not be detected after anaerobic incubation of blood and dopa, the appearance of the pressor amine is exclusively due to an enzyme present in the kidney itself.

Holtz claimed (5) that the liver and the small intestine of guinea pigs contain the dopa-decarboxylase which is the active catalyst in the formation of hydroxytyramine from dopa. Both the liver and the intestine of the cat, perfused with blood containing dopa under conditions of reduced venous outflow, failed to form the pressor amine. Therefore, the perfused cat's kidney is distinguished from the intestine and liver in its ability to form hydroxytyramine.

SUMMARY

1. The production of a pressor substance, presumably hydroxytyramine, by decarboxylation of dopa occurs in extracts of guinea-pig's kidneys under conditions of oxygen lack.

2. A similar reaction takes place in ischemic cat's kidneys perfused with blood containing dopa.

3. The amount of the pressor substance produced in the perfused kidney depends on the rate of flow through that organ.

4. Perfusates of liver and gut of the cat, produced under analogous conditions, as well as cat's blood incubated with dopa, have no pressor properties.

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THE RELATIONSHIPS BETWEEN TOTAL, ACID, AND NEUTRAL CHLORIDES OF GASTRIC JUICE

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A number of theories have been advanced to account for the variation in the composition of gastric juice. Pavlov (1) claimed that gastric juice as it flows from the gastric glands has a constant acidity which may be modified to some extent by admixture with alkaline mucus. Roseman (2) on the other hand maintained that gastric juice as it flows from the gastric glands has a constant total chloride concentration; variations in the acidity of the juice are due to the conversion of a variable proportion of neutral chloride to acid chloride by the parietal cells. Hollander (3) has concluded that gastric juice consists of a variable mixture of a pure isotonic solution of HCl secreted by the parietal cell and an alkaline secretion of constant composition containing all the other elements of the juice. On the other hand, Liu, Yuan and Lim (4) believe that gastric juice consists of a mixture of three components; *a*, a pure hypertonic solution of HCl; *b*, an alkaline, chloride-free fluid secreted at a constant rate, and *c*, a neutral chloride fluid secreted at a rate proportional to that of the acid component.

The above theories are based on observations or assumptions regarding the behavior of the various chloride fractions in gastric juice. The contradictory and incompatible nature of the theories is the result of serious disagreement about these fundamental observations and assumptions. It was hoped, therefore, that a careful study of the variation in the chloride fractions as they are related to one another and to the rate of secretion would permit a choice between the theories or clearly define a new interpretation.

METHODS. Dogs with pouches of the entire stomach (vagotomized) were used in this study. Special measures have been taken to minimize certain sources of error in the collection of gastric juice. The most important innovation consisted in maintaining a continuous and uniform rate of secretion by repeated subcutaneous injections of histamine at ten minute intervals. In order to produce different rates of secretion the dosage of histamine was changed from time to time and from day to day,

so that the scale of volume-rate ranging from 5 to 35 cc. per twenty minutes was covered several times. By this technique the volume of juice secreted in a given period was an accurate measure of the rate of secretion which persisted throughout that period. Samples were collected at twenty minute intervals so that opportunity for variations in secretory rate were still further limited.

Errors due to irregular flow from and temporary retention of juice within the pouch were controlled by two methods. First, the high rates of secretion obtained provided a large enough volume so that regular flow was promoted and the effect of retention of small quantities of juice reduced. Secondly, the device used for the collection of juice assured complete collection without possibility of obstruction of the flow.

In every experiment, samples of gastric juice were not collected for analysis until after a profuse flow of secretion had been stimulated during the first hour and the resulting juice discarded. This flow served completely to flush accumulated mucous secretion from the stomach. This precaution has not been observed by previous experimenters.

Two dogs were used once a week for eight weeks, providing 100 samples of gastric juice from each. In addition 183 samples were collected from six dogs on two successive days and pooled at the time of collection according to the volume-rate of secretion. Nine pooled samples were thus obtained with the average volume-rate of the lowest 6.0 cc. and of the highest 29.1 cc. per twenty minutes. The large number of observations was the final assurance that the errors would be minimal in the final results.

Aliquots of each sample of gastric juice were taken for total chloride determination by the method of Na_2CO_3 fusion followed by Volhard titration and for titration of total acidity, using phenolphthalein as indicator. Neutral chloride was calculated as the difference between total and acid chloride. All analyses were done in duplicate and repeated, if necessary.

Statistical methods were employed in the analysis of the data for two reasons. First, it was essential to know the reliability of the results, and second, the only accurate method of discovering the nature of a relationship which may exist between two variables is to determine by statistical means the line which best fits the plotted points. The usual statistical methods for curve fitting yield two lines, known as regression lines. These are obtained, in principle, by allowing each of the variables in turn to be independent and assuming that the failure of points to fall on the theoretical line is due to variation in the dependent variable only. If the points are widely scattered, the two regression lines may be quite dissimilar. Under such circumstances one may obtain a single, mutual regression line which places equal responsibility on the two variables for failure of the points to fall on the theoretical line. In this investigation, when the ordinary regression lines have given widely differing predictions, mutual

regression lines have also been fitted. In these cases, the obviously divergent predictions have been excluded from the final averages.

For the statistical procedures involved in fitting ordinary regression lines (linear or curvilinear) the principles outlined by Schultz (6) were employed. Since the authors are not aware of any published account of the method of fitting a mutual regression line, the formulas for that purpose are given here.[1] The mutual regression equation may be written:

$$A + BX - Y = O,$$

where A and B are constants determined by statistical methods and are given by the following formulas:

$$B = -K \pm \sqrt{K^2 + 1}, \qquad A = M_y - BM_x,$$

in which $K = \frac{\sigma^2_x - \sigma^2_y}{2\sigma_x \sigma_y r_{xy}}$ and M_x, M_y are the means of the respective variables, the σ's their standard deviations, and r_{xy} their correlation coefficient. There are two values for B—one gives the best fitting line and the other the worst—but the value to be taken in any practical case is obvious from the nature of the data. The standard errors of the constants A and B are not known, but the standard error of estimate, S, in predicting either X or Y may be obtained from the formula,

$$S^2 = \tfrac{1}{2}(\sigma^2_x + \sigma^2_y) - \tfrac{1}{2}\sqrt{(\sigma^2_x - \sigma^2_y)^2 + 4\sigma^2_x \sigma^2_y r^2_{xy}}$$

Results. *Volume-output method.* When the output of either total chloride, acid chloride, or neutral chloride is plotted against the volume-rate of secretion, a linear relationship immediately becomes apparent. This is shown in figure 1, in which the raw data obtained from the pooled samples are plotted together with the statistically-fitted regression lines. The statistical data, including correlation coefficients, the constants in the regression equations, standard errors of these constants, and the standard errors of estimate are presented in table 1. The correlation coefficients are remarkably high.[2] The standard error of the *b* constant, or slope, is very small in each case. With the exception of the equations relating the output of neutral chloride to the volume-rate of secretion, the two ordinary regression equations yield almost identical predictions.

The important fact to be noted is that *as the volume-rate of secretion increases, the outputs of total chloride, acid chloride, and neutral chloride all*

[1] These formulas were taken from a series of lectures by Professor Schultz at the University of Chicago.

[2] This is due to the repetition of the variable, volume-rate, i.e., the output figures were all obtained by multiplying concentration by volume-rate, and this product was then correlated with the volume-rate. This spurious correlation, however, only augments a pre-existing correlation.

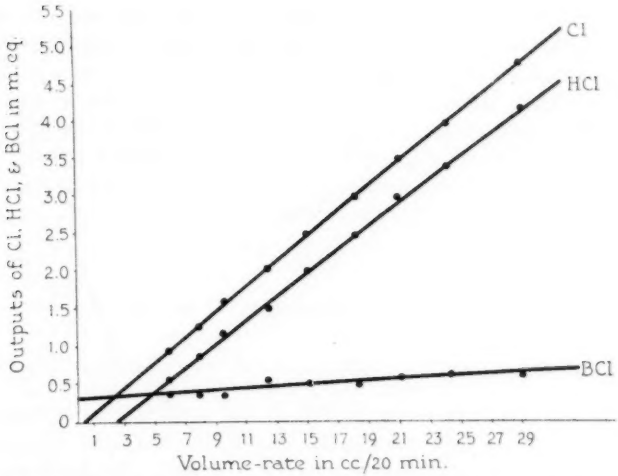

Fig. 1. Relationships between the volume-rate of secretion and the outputs of the chloride fractions for the "pooled samples." (Volume-output method.)

TABLE 1

Linear relationships between volume-rate and outputs of chloride fractions

(Volume-output method)

	DOG 1			DOG 2			POOLED SAMPLES		
	Cl	HCl	BCl	Cl	HCl	BCl	Cl	HCl	BCl
	Correlation coefficient								
	0.999	0.996	0.541	0.999	0.997	0.783	0.999	0.999	0.928
	Volume independent*								
a	−0.0715	−0.2613	0.1899	−0.0632	−0.2118	0.1486	−0.0527	−0.3550	0.3009
b	0.1667	0.1575	0.00921	0.1657	0.1518	0.0139	0.1649	0.1535	0.0115
σ_b	0.00086	0.00105	0.00166	0.00037	0.00078	0.00112	0.00088	0.00142	0.00175
S_x	0.0455	0.0554	0.0880	0.0352	0.0699	0.0752	0.0172	0.0277	0.0341
	Volume dependent†								
a'	0.4645	1.1766	−14.28	0.3976	1.4756	−22.60	0.3336	2.1800	−23.13
b'	5.9835	6.2946	31.92	6.0293	6.5529	44.10	6.0590	6.5780	80.73
$\sigma_{b'}$	0.0308	0.0419	5.01	0.0136	0.0329	3.54	0.0324	0.0610	12.29
S_v	0.272	0.350	4.49	0.213	0.459	5.59	0.104	0.181	2.76

* The regression of Cl, HCl, or BCl (designated by x) on volume-rate (V) has the following form:

$$X = a - bV.$$

The standard error of estimate of X is denoted by S_x and the standard error of b, the slope of the line, by σ_b.

† The regression of V on X is given by $V = a' + b'X$, and the standard errors are designated similarly to the preceding case.

increase in a linear fashion, but at different rates. The slope of the total chloride line is significantly greater than that of the acid chloride line, and hence the two lines are divergent; consistent with this is the fact that the output of neutral chloride increases with the volume-rate of secretion.

From the volume-output equations one can predict the relationship existing between the rate of secretion and the concentration of the chloride fractions of the gastric juice. For example, the equation relating the total chloride output to the volume-rate has the following form:

$$Cl = a + bV.$$

By dividing this equation by volume-rate, V, we obtain, $\frac{Cl}{V} = \frac{a}{V} + b$, in which $\frac{Cl}{V}$ represents the *concentration* of total chloride. This equation has the form of a hyperbola, in which the constant, b, represents the asymptotic value. This asymptote reveals the maximal concentration of total chloride which the gastric juice can attain. The same procedure can be applied to the equations for acid chloride and neutral chloride outputs. Predictions made by this method for the maximal concentrations of total and acid chlorides and the minimal concentration of neutral chloride will be found in table 4. From this table one can also see the close agreement between the predictions from the two ordinary regression lines.

Since algebraic manipulations of regression equations may introduce further inaccuracies in predictions, it is desirable to investigate *directly* the relationship between the volume-rate of secretion and the concentrations of the chloride fractions. The above discussion, however, serves to indicate that the relationships are hyperbolic in form and that hyperbolic regression equations should be fitted to the data.

Volume-concentration method. When the concentrations of total chloride, acid chloride, or neutral chloride are plotted against the volume-rate of secretion, hyperbolic relationships become evident. This is shown in figure 2, in which the raw data obtained from the pooled samples are plotted together with the statistically-fitted regression curves. The correlation indexes and other statistical parameters are presented in table 2. The correlation indexes are lower than the correlation coefficients of the previous method; since the volume-rate and the concentrations of the chloride fractions were determined independently, spurious correlation is not present. Only one hyperbolic regression curve has been fitted to each set of observations.[3]

[3] Since the observations are spread out along the horizontal part of the curve, only the regression equations which are obtained by minimizing the deviations in the vertical direction are of significance in this case.

It should be noted that *as the volume-rate of secretion increases, the concentrations of total chloride and acid chloride increase in a hyperbolic fashion,*

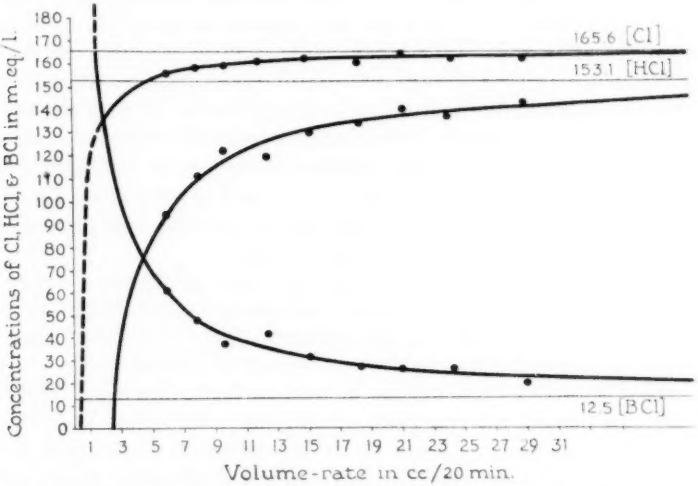

Fig. 2. Relationship between the volume-rate of secretion and the concentrations of the chloride fractions for the "pooled samples." (Volume-concentration method).

TABLE 2

Relationships between the volume-rate and the concentration of the chloride fraction

(Volume-concentration method)
Volume independent*

	DOG 1			DOG 2			POOLED SAMPLES		
	[Cl]	[HCl]	[BCl]	[Cl]	[HCl]	[BCl]	[Cl]	[HCl]	[BCl]
	Correlation index								
	0.522	0.696	0.590	0.554	0.779	0.727	0.955	0.987	0.978
c	−63.99	−230.96	166.97	−40.61	−180.76	140.15	−62.98	−349.36	286.38
d	166.09	155.19	10.90	164.04	149.71	14.33	165.57	153.05	12.52
σ_d	0.90	2.04	1.95	0.58	1.38	1.26	0.72	1.93	2.06
$S_{[x]}$	2.99	6.79	6.52	2.83	6.74	6.13	0.88	2.36	2.52

* The hyperbolic regression of [Cl], [HCl], or [BCl], designated by $[x]$, on V is written in the form:

$$[x] = \frac{c}{V} + d.$$

whereas the concentration of neutral chloride decreases in a hyperbolic fashion. Since these relationships are hyperbolic in form, the asymptotes, or b con-

stants, reveal the limiting concentrations of the chloride fractions of gastric juice. The maximal concentration of total chloride varies from 164.0 to 166.1 milli-equivalents per liter. The maximal concentration of acid chloride varies from 149.7 to 155.2 milli-equivalents per liter. The minimal concentration of neutral chloride varies from 10.9 to 14.3 milli-equivalents per liter. The consistency of the results is shown by the fact that the limiting values for these elements are not significantly different from dog to dog. The difference between the maximal total chloride and acid chloride concentration is statistically different in each case. *The fact that the minimal concentration of neutral chloride in each case is positive and significantly*[4] *different from zero proves that even at infinitely high rates of secretion neutral chloride is present in the gastric juice.*

A remarkably close agreement exists between the limiting values determined directly by the volume-concentration method and those indirectly predicted from the volume-output method. This comparison can best be made by consulting the first three columns of table 4.

Another important relationship is evident from figure 2. The total chloride and neutral chloride lines cross at the point where the acidity of the juice becomes zero. This of course means that in the absence of acid all the chloride is present in the form of neutral chloride. Upon further extrapolation to the left (fig. 2) the neutral chloride line crosses and extends above the total chloride asymptote, implying hypertonic gastric juice; similarly the total chloride line takes on negative values. Obviously extrapolation in this direction leads to conclusions which are absurd from the standpoint of physiology, although Liu, Yuan and Lim based their theory on this unwarranted procedure.

Concentration-concentration method. When the concentration of either total chloride or neutral chloride is plotted against the acidity of gastric juice, a linear relationship becomes apparent. This is shown in figure 3, in which the raw data obtained from the pooled samples are plotted together with the statistically fitted mutual regression lines. The statistical results are presented in table 3. In relating the acidity to the neutral chloride concentration higher[5] correlation coefficients were obtained than in relating the acidity to the total chloride concentration. In cases where the correlation coefficients were low, predictions from the two regression equations differed considerably, hence mutual regression lines were also fitted to the data.

It should be noted that *the acidity of gastric juice varies directly with the*

[4] The minimal concentration of neutral chloride is more than three times its standard error, or more than approximately 4.5 times its probable error.

[5] This is again due to spurious correlation resulting from the fact that the neutral chloride concentrations were determined by subtracting the acid chloride from the total chloride.

total chloride concentration and inversely with the neutral chloride concentration. From the equations representing these relationships one can calculate the maximal total or acid chloride concentration at the point where neutral chloride is assumed to be absent. Taking only the more reliable estimates, the maximal acid or total chloride concentration obtained by this method varies from 163 to 168 milli-equivalents per liter, as shown in table 4. *By this method the maximal acidity of gastric juice is found to be significantly higher than by the volume-concentration method. This discrepancy results from the assumption that neutral chloride can be absent from gastric juice.*

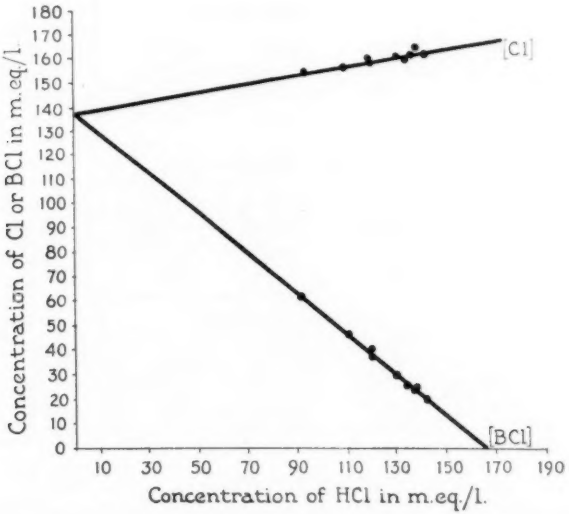

Fig. 3. Relationships between the concentration of the chloride fractions for the "pooled samples". (Concentration-concentration method.)

Discussion. It has been claimed (2) that the total chloride concentration of gastric juice is constant, but the results obtained by ourselves as well as others (3, 4, 7) demonstrate very definitely that it varies both with the rate of secretion and the acidity. This finding is opposed to the theory of Rosemann for the variations in the composition of gastric juice. This behavior of the total chloride has been frequently overlooked because the range of variation is small and noticeable only at the slower rates of secretion.

Pavlov (1) claimed that if accumulated mucus is first flushed from a gastric pouch, the acidity remains constant. Hollander (3) has reported

the same if the juice is collected in such a way as to prevent irritation and consequent mucus production. On the other hand we have found that

TABLE 3

Linear relationships between the concentration of the chloride fraction

(Concentration-concentration method)

	DOG 1		DOG 2		POOLED SAMPLES	
	[Cl]	[BCl]	[Cl]	[BCl]	[Cl]	[BCl]
	Correlation coefficient					
	0.552	−0.932	0.647	−0.957	0.871	−0.993
	[HCl] independent*					
a	132.95	132.93	133.10	133.08	138.43	138.41
b	0.2050	−0.7948	0.2047	−0.7952	0.1766	−0.8232
σ_b	0.0312	0.0313	0.0244	0.0243	0.0375	0.371
$S_{[x]}$	2.92	2.93	2.59	2.59	1.45	1.44
	[HCl] dependent†					
a'	−102.69	163.23	−193.78	164.64	−564.66	167.53
b'	1.4876	−1.0923	2.0460	−1.1520	4.2966	−1.1976
σ_b'	0.227	0.0429	0.244	0.0353	0.916	0.0539
$S_{[HCl]}$	7.88	3.43	8.19	3.12	2.42	1.73
	Mutual regression‡					
A	130.16	139.04	131.46	136.94	138.22	139.05
B	0.2254	−0.8468	0.2168	−0.8238	0.1783	−0.8282
C	2.86	2.21	2.54	2.01	1.45	1.10

* The regression equation has the form:

$$[x] = a + b[HCl],$$

where $[x]$ stands for $[Cl]$ or $[HCl]$. The remaining symbols are used as in previous tables.

† The regression equation with $[HCl]$ as dependent variable is written as follows:

$$[HCl] = a' + b'[x].$$

‡ The symbols employed in the mutual regression equations are the same as those in the equations under methods with $x = [HCl]$ and $y = [Cl]$ or $[BCl]$. For example, the first equation can be written out as follows:

$$130.16 + 0.2254[HCl] - [Cl] = 0.$$

the acidity varies with the rate of secretion, even when accumulated mucus has been flushed from the pouch. The discrepancy is only superficial, for the hyperbolic form of the acidity curve permits wide fluctuations in high rates of secretion without significant changes in acidity (fig. 2). If

TABLE 4

Summary of limiting values for chloride fraction

	VOLUME-OUTPUT METHOD		VOL.-CONC. METHOD	CONCENTRATION-CONCENTRATION METHOD					
				[HCl]-[Cl] relationship			[HCl]-[BCl] relationship		
	Volume independ.	Volume depend.	Volume independ.	[HCl] independ.	[HCl] depend.	Mutual	[HCl] independ.	[HCl] depend.	Mutual
Maximal [Cl]									
Dog 1	166.7 ±0.86	167.2 ±0.87	166.1 ±0.90	167.2±2.92	210.6±7.88	168.0±2.86	167.2±2.93	163.2±3.43	164.2±2.21
Dog 2	165.7 ±0.37	165.8 ±0.37	164.0 ±0.58	167.3±2.59	185.3±8.19	167.9±2.54	167.3±2.59	164.6±3.12	166.2±2.01
Pooled samples	164.9 ±0.88	165.0 ±0.88	165.6 ±0.72	168.1±1.45	171.3±2.42	168.2±1.45	168.1±1.44	167.5±1.73	168.0±1.10
Maximal [HCl]									
Dog 1	157.5 ±1.05	158.9 ±1.06	155.2 ±2.04	Same as above					
Dog 2	151.8 ±0.78	152.7 ±0.77	149.7 ±1.38						
Pooled samples	153.5 ±1.42	152.0 ±1.41	153.1 ±1.93						
Minimal [BCl]									
Dog 1	9.17±1.66	31.35±4.92	10.90±1.95	0	0	0	0	0	0
Dog 2	13.9 ±1.12	22.68±1.82	14.33±1.26	0	0	0	0	0	0
Pooled samples	11.49±1.75	12.39±1.89	12.52±2.06	0	0	0	0	0	0

mucus production is minimized, the secretion rates must obviously be still lower before they affect the acidity.

By the two "volume" methods the maximal total chloride concentration averaged 165.7 m.eq./l, a value which is isotonic as would be demanded by the observations of Gilman and Cowgill (9). By similar methods Liu, Yuan and Lim obtained 176 for the dog and Wang 158.6 for man. The high and probably incorrect value of 176 may be due to the state of the dogs or to faulty methods for determining total chloride, but the other possibilities suggested by Hollander (8) do not apply. There appears to be no significant discrepancy between the various estimates for maximal acidity obtained by the "volume" methods (table 5). Of most significance is our confirmation of Liu et al., and Wang that the neutral chloride concentration does not fall to zero. Neither we nor any other investigators have observed samples of gastric juice free of neutral chloride; these results show that even at infinitely rapid rates of secretion neutral chloride will still be present.

TABLE 5

Final average values

All values as milli-equivalents per liter

	VOLUME METHODS			CONCENTRATION-CONC. METHOD	
	Authors' Dog	Liu et al. Dog	Wang Man	Authors' Dog	Hollander Dog
Maximum total chloride conc.	165.7	176.0	158.6	166.9	165.2
Maximum acidity	153.8	157.7	139.5	166.9	165.2
Minimal neutral chlor. conc.	12.1	18.3	26.8	0	0
Osmotic press. of blood		168	159		

Of the several theories which have been advanced to explain the variations in the composition of gastric juice, none seems to fulfill the requirements set by the available observations. The insurmountable objection to Rosemann's theory has been mentioned. The theory of Liu, Yuan and Lim, which assumes that the gastric glands secrete a chloride-free fluid, was based on the unjustified extrapolation of the chloride hyperbola pointed out under Results.

Hollander's theory is also based on an extrapolation that is questionable. He assumed that the parietal cell secretes no neutral chloride and justified this assumption by demonstrating that its use led to an acceptable value for the acidity of the parietal secretion. From the straight line relating the concentrations of acid and neutral chloride, Hollander found that the intercept corresponding to zero concentration of neutral chloride represented 165.2 m.eq./l. of acid. The same procedure applied to our data (concentration-concentration method) yields 166.9 m.eq./l.

It should be noted, however, that this figure is significantly greater than the maximal acidity obtained from the acidity hyperbola and slightly, but uniformly, greater than the maximal total chloride concentration obtained similarly. This is, of course, the inevitable consequence of assuming the neutral chloride concentration to be zero, as Liu, Yuan and Lim insisted in their rejection of Hollander's method.

The objection, therefore, to Hollander's theory is that it maintains that neutral chloride can be absent from gastric juice, which is contrary to all observation.

Before one can advance any theory to account for the variations in the composition of gastric juice, a fundamental question must be answered. *Why is neutral chloride always present in gastric juice?* Is it because the parietal cells secrete neutral chloride, or, is it because an increase in the activity of the parietal cells is accompanied by an increased secretion of neutral chloride by non parietal cells? Since the present analysis of the behavior of only the chloride fractions provides no answer to this question, no theory can as yet be constructed to account for the composition of gastric juice.

CONCLUSIONS

1. The relationships between the various chloride fractions of gastric juice have been investigated by a statistical analysis of data obtained from 383 samples of gastric juice carefully collected from dogs with vagotomized pouches of the entire stomach. The continuous secretion of gastric juice at various rates was maintained by subcutaneous injections of histamine at ten minute intervals.

2. As the volume-rate of secretion increases, the *outputs* of total, acid, and neutral chlorides all increase in a linear fashion, but at different rates.

3. As the volume-rate of secretion increases, the *concentrations* of total and acid chlorides increase in a hyperbolic fashion, whereas the concentration of neutral chloride decreases in a hyperbolic fashion.

4. As the acidity of gastric juice increases, the total chloride concentration increases and the neutral chloride concentration decreases, both in a linear fashion.

5. From the statistically fitted equations representing the above relationships it has been found that at infinite rates of secretion the maximal total chloride concentration of gastric juice is 166 milli-equivalents per liter, the maximal acidity, 154 milli-equivalents per liter, and the minimal neutral chloride concentration, 12 milli-equivalents per liter.

6. None of the various theories advanced to explain the variations in the composition of gastric juice are wholly acceptable in the light of the above findings.

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OBSERVATIONS ON THE WATER INTAKE IN AN ADULT MAN WITH DYSFUNCTIONING SALIVARY GLANDS[1]

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The theory of Cannon (1) that the salivary glands are essential to thirst and control of water uptake in man has been questioned in recent years by different investigators (2), (3), (4), (5). Dill (6) in a critical review of the literature concludes that the original stimulus of thirst probably is located in the muscle cells and is brought about when an upset in the electrolytic balance occurs. Although the experimental evidence opposing Cannon's view may be correct and justified, it should be pointed out that in these recent studies comparisons are being made between results obtained from normal human beings and from animals which, in many cases, had previously been prepared by operation for such experimental purposes. It is also true that the experiments of Cannon on man were carried out over relatively short periods of time and under rather acute conditions, while the work on animals in many cases was made several days or months after the animal had become adjusted to his new environmental state. For obvious reasons many of the experiments carried out on animals could hardly be duplicated on man, although it would be the ideal way to make accurate comparisons.

It was our good fortune to come upon a University student who claims that he has been without salivary secretions since earliest infancy. A medical examination confirmed a complete absence of all salivary glands and ducts. Likewise, no change in mouth secretions was recorded following subcutaneous injections of 6.0 mgm. of pilocarpin. Although the mucous membranes of the mouth were always dry, there was evidence of a certain amount of mucinous secretion from gland cells along the inside of the cheeks and under the tongue. Upon testing these secretions with starch paste, there was no evidence of the presence of amylolytic enzymes (7).

The subject also reports a deficiency of lachrymal secretion, as well as the early onset of dental caries, so severe that false dentures were needed

[1] This investigation was aided materially by the donation of funds from the Graduate School of the University of Illinois for which grateful acknowledgment is made.

at the age of 21 years. Similarly, the recent experiments of Cheyme (8) show that rats without salivary glands show a greater frequency of dental caries. His dry mouth condition does not interfere with his sleep. Adolph (4) has also observed that the sleep of dogs with esophageal fistulae is not interrupted if the water deficiency is of a mild degree.

The subject has learned to relieve the dry uncomfortable feeling in his mouth by taking a few swallows of water nearly every hour. Although at times he can go without water for two hours, this occurs very infrequently. Along with the frequent intake of small quantities (60 cc.) at approximately hourly intervals, he becomes actually thirsty about four times a day when his consumption is about 250 cc. per draft. A simple calculation of the total water intake per day, excluding water in the food, would give him 1960 cc. assuming that he drinks small quantities of 60 cc. on 16 hourly occasions. This calculated water intake agrees closely with previously reported experiments (9) in which a comparative study of the total fluid intake of this subject and four normal persons was made over a period of 18 days. The average daily intake for the four control subjects was 1740 cc., while the total fluid intake, which included coffee, tea and milk, gave an average figure of 2615 cc. During the same period, the experimental subject showed such average quantities as 1975 and 2783 cc. respectively. Since Richter (10) has recently reported that the amount of fluid intake in man and animals is related to body size, we have recalculated our data on the basis of surface area and found the amounts to vary from 1,145 to 1,476 cc. per square meter, with the exception of one subject in the control group, who habitually drank extremely large quantities of tea and coffee each day, the subject without salivary glands consuming 1,421 cc. per sq.m. of surface area. Judging from these experiments it would seem that the total daily fluid intake is not materially affected by the lack of salivary glands as registered by the experimental subject.

It is a well known fact that a normal person with a dry mouth has a pronounced desire to drink. Therefore, we decided to induce this type of thirst in normal individuals in order to determine the amount of fluid they would require to relieve their discomfort, and then compare their intake with that of the experimental subject. Nine normal individuals were allowed to drink as much water as they desired to satisfy their thirst, and then, immediately after, the mucous membranes of their mouths were exposed to a stream of warm, dry air until they became very thirsty. Water was supplied in calibrated containers and the subjects were instructed to drink until their thirst was relieved. It was interesting to note that the average intake for the nine subjects was 88 cc. Later experiments, in which the subjects were instructed to drink slowly and only just enough to satisfy their thirst, gave practically the same results. On the basis of

60 cc. per draft in the experimental subject, this was 47 per cent greater. In no case did the mucous membranes of the mouths of the controls appear as dry as those of the experimental subject.

To find whether there was any possible difference in body water metabolism levels between the experimental and normal subjects, experiments were made in which they were given rather large quantities of sodium chloride by mouth, after which a record was made of water intake, urine production, and the amount of salt excreted in the urine. This, according to Coller and Maddock (11), would give us information concerning the physiological normalcy of the experimental subject; also it would show whether an actual desire or thirst for water could be elicited in him.

The experimental procedure was as follows: on five different occasions the experimental subject and two controls were given 10 grams of sodium chloride in 100 cc. of water by mouth, about 12 to 14 hours after their last meal. Hourly records of water intake, urine output, and excretion of urine chlorides (Volhard-Harvey method) were made for a period of six hours. We chose this length of time because Adolph (4) had reported that 2 to 5 hours are necessary to make body adjustments following a water deficit in man. No restrictions or record of water intake were made up to the time of the experiment. Each subject reported a definite sensation of thirst immediately following the ingestion of the salt solution and responded by drinking rather large quantities of water. In the first three of the five experiments performed (period I, table 1), in which the subjects were allowed to drink ad libitum, it was noted that the controls drank 42 per cent more water than the experimental subject and also excreted 51 per cent more urine during the time of the experiment. Adolph (4) also reports that dogs, made deficient in water, exceed the expected water intake by 17 per cent when allowed to drink freely. In the last two experiments (period II, table 1) the experimental subject served as a control for the frequency of drinking. Thus, whenever he expressed the desire to drink the two controls were requested to do likewise. It is interesting to note that in this type of experiment the controls again drank more water and excreted more urine than the experimental subject. The actual increase in water intake was 46 per cent and of urine excretion 35 per cent. Table 1 shows that the water intake and urine output were higher in period II than in period I in both control and experimental subjects. This was probably due to the fact that the atmospheric temperature was higher than when the former experiments were performed. The average amounts in both the water intake and the urine output of the controls in the two periods were calculated to be 44 per cent and 42 per cent above those of the experimental subject, but it is noteworthy that the amounts of chloride excreted were the same for all three individuals.

The observation that the water intake in the control subjects was 44

per cent above the experimental subject indicates that the normal individual habitually drinks more water than his body requires. Further, since the ratio between water intake and the urine excretion of the experimental subject is practically the same as that of the controls, and since the amounts of salt excreted are the same, we feel justified in drawing the conclusion that our subject, with the exception of not possessing salivary glands, is physiologically normal.

TABLE 1

The effects of NaCl ingestion on water intake and urine and salt excretion

	TWO CONTROL SUBJECTS				EXPERIMENTAL SUBJECT			
	Time	Water intake	Urine output	Total Cl in urine	Time	Water intake	Urine output	Total Cl in urine
		cc.	*cc.*	*grams*		*cc.*	*cc.*	*grams*
Period I. Average of 3 experiments	1 hr.	323	59	0.378	1 hr.	247	39	0.486
	2 hr.	258	65	0.889	2 hr.	263	64	0.979
	3 hr.	168	81	1.393	3 hr.	102	77	1.203
	4 hr.	190	84	1.000	4 hr.	98	62	0.979
	5 hr.	98	87	0.809	5 hr.	72	46	0.752
	6 hr.	198	119	0.875	6 hr.	88	39	0.687
	Total	1,235	495	5.344		870	327	5.086
Period II. Average of 2 experiments	1 hr.	633	44	0.409	1 hr.	593	36	0.582
	2 hr.	214	59	0.784	2 hr.	198	69	1.041
	3 hr.	384	78	1.177	3 hr.	168	73	1.239
	4 hr.	233	93	1.183	4 hr.	100	75	1.280
	5 hr.	188	112	0.891	5 hr.	80	73	1.230
	6 hr.	173	146	0.816	6 hr.	110	68	1.222
	Total	1,825	532	5.260		1,249	394	6.594
Average of 5 experiments		1,530	514	5.302		1,060	361	5.840

We realize that our findings have not solved the problem of the true site of the thirst sensation, nor do we venture to advance any new theory concerning it. However, we believe that our observations on an adult man without salivary glands will help to bridge a gap between the different types of experiments conducted by previous investigators. Our results, as well as those reported by others on dogs (2), (3), (4), (5), show quite conclusively that the salivary glands, while essential in keeping the mouth moist and comfortable, are not the sole factors governing thirst. Furthermore, we have noted that in all cases where thirst was induced either by drying of the mouth or by the ingestion of sodium chloride, the normal individuals consumed at least 44 per cent or more water than the experi-

mental subject. Although this may be related to the presence or absence of salivary glands it would seem more logical to explain this on the basis of the drinking habits of the normal individual. This extra flushing of the body probably has a salutary effect; but it is evident that through control the water intake can be greatly reduced without upsetting the normal functioning level of water in the body.

CONCLUSIONS

1. The absence of salivary glands in man does not cause him to drink greater quantities of fluid than a normal individual.

2. Normal subjects, when made thirsty by artificial means such as exposure of the mouth to dry heat or ingestion of sodium chloride by mouth, consume from 42 to 47 per cent more water to moisten the mouth than the experimental subject habitually drinks.

3. A person without salivary glands may have a normal water and salt metabolism.

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THE ACTION OF DESOXYCORTICOSTERONE ACETATE AND PROGESTERONE ON THE BLOOD AND TISSUE CHLORIDES OF NORMAL AND ADRENALECTOMIZED ANIMALS

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In a short note we recently reported (1) that desoxycorticosterone acetate (D.C.A.) given in relatively high doses decreases the blood chloride concentration in the rat. In adrenalectomized animals whose blood chlorides are below normal, D.C.A. exerts the opposite effect. Continuing our studies of this phenomenon we wished to establish whether the same dose of D.C.A. which raises the blood chlorides in the adrenalectomized animal can lower them in the presence of the adrenals. This appeared to be important in order to determine whether the latter action is merely the result of overdosage, obtainable both in the presence and in the absence of the adrenals as long as sufficiently large doses are given, or whether the hypochloremic action is dependent upon the presence of functional adrenal tissue. We hoped furthermore that urine and tissue chloride determinations would help us to understand the mechanism through which D.C.A. decreases the blood chloride concentration in the intact rat. At the same time we wished to investigate the effect of progesterone on the blood chlorides of the adrenalectomized rat since this compound differs from D.C.A. only in that it has no —OH group on C atom 21 and resembles the latter in its pharmacological actions. Both compounds maintain adrenalectomized animals alive, cause progestational proliferation of the endometrium and development of the mammary glands. Yet unlike D.C.A., progesterone does not produce any significant decrease in the blood chloride concentration of the intact rat (2). In view of these observations, additional comparative studies concerning the pharmacological actions of progesterone and D.C.A. appeared necessary to obtain further information concerning the physiological significance of the C 21 hydroxyl group.

METHODS AND MATERIALS. Our first experiments were performed on young adult male and female albino rats, the various groups having an average body weight of 140 to 174 grams. The females were somewhat lighter than the males in most cases. Later we noted, however, that still younger rats are even more suitable for such experiments since they show more rapidly developing and pronounced signs of adrenal insufficiency.

Therefore rats weighing only 80 to 84 grams on the average were used exclusively in our second experimental series.

The adrenalectomies were performed from the dorsal route under ether anesthesia. In the first two experimental series (tables 1 and 2) progesterone or D.C.A. was administered in doses of 5 mgm. twice daily subcutaneously in 0.2 ml. of peanut oil. The injections were started on the day of operation and continued for two days, the last injection being given 6 hours before sacrificing the animals. The slightly different method of treatment used in the last series (table 3) is described in the experimental section. Before every chemical determination, food was withdrawn for 24 hours so as to avoid post-absorptive changes. The animals were killed without anesthesia by cutting the carotid artery and jugular vein.

TABLE 1

Blood and tissue chlorides in adult rats

TREATMENT	WHOLE BLOOD	P	PLASMA	P	MUSCLE	P	BODY WEIGHT	SEX
Intact controls	419		557		135		156	Female
Intact D.C.A.-treated	378	<0.01	572	0.2	146	0.5	154	Female
Adrenalectomized	381	<0.01	563	0.5	160	<0.01	140	Female
Adrenalectomized D.C.A.-treated	397	0.2	561	0.6	189	<0.01	154	Female
Adrenalectomized progesterone-treated	382	0.7	534	0.05	150	0.4	148	Female
Intact controls	407		562		134		157	Male
Intact D.C.A.-treated	378	0.05	556	0.5	138	0.5	174	Male
Adrenalectomized	370	0.05	520	<0.01	135	0.5	155	Male
Adrenalectomized D.C.A.-treated	383	0.1	581	<0.01	193	<0.01	161	Male
Adrenalectomized progesterone-treated	365	0.6	487	0.5	152	0.3	170	Male

All the blood was collected in crucibles using potassium oxalate to prevent clotting. The amount of blood thus collected was accurately measured in the second experimental series (see table 2) because it gives a good indication of the volume of freely circulating blood. Accurate total blood volume determinations are not practicable in animals of this size without perfusion of the vascular system and this would interfere with the blood and tissue analyses. It must be kept in mind however, that the blood volume thus determined is considerably below the total blood volume because much of the blood which stagnates in capillaries and small veins is not recovered.

The tissue chlorides are expressed as milligrams of NaCl/100 ml. of blood or 100 grams of tissue. They were determined on 1 gram samples

by the Van Slyke (3) method and the whole blood and plasma chlorides by a modification of the above technique in which the concentration of the digestion mixture was adjusted for micro-determination on 0.2 ml. samples. The blood sugars are expressed as mgm/100 ml. and were determined by the Somogyi (4) modification of the Shaffer-Hartmann technic.

The significance of the apparent differences between the treated and control groups was evaluated by "Student's" method for small samples (5) and is expressed in the tables in terms of probability, estimated by graphic interpolation in Fisher's table of t. In accordance with the usual convention, differences between series cannot be accepted as significant when P is greater than 0.05. In calculating the significance of the apparent changes the intact untreated animals served as controls for the

TABLE 2

Blood and tissue chlorides, blood glucose and blood volume in young male rats

TREATMENT	WHOLE BLOOD CHLORIDES	P	PLASMA CHLORIDES	P	MUSCLE CHLORIDES	P	BLOOD VOLUME	P	BLOOD GLUCOSE	P	BODY WEIGHT
Intact controls	416		560		138		1.5		84		80
Intact D.C.A.-treated	357	<0.01	522	<0.01	155	0.05	1.6	0.7	57	<0.01	81
Adrenalectomized	379	<0.01	507	<0.01	142	0.6	0.8	<0.01	43	<0.01	84
Adrenalectomized D.C.A.-treated	417	<0.01	553	<0.01	169	<0.01	1.4	<0.01	61	0.02	81
Adrenalectomized progesterone-treated	386	0.5	516	0.5	139	0.5	0.8	0.6	44	0.6	83

intact D.C.A. injected groups while the determinations on adrenalectomized-treated animals were compared with those on untreated adrenalectomized controls.

EXPERIMENTAL RESULTS. The results of our experiments are summarized in the following tables which are self-explanatory and do not require much comment. Each figure represents the average of six individual determinations on six different animals. The results in table 2 are a repetition and confirmation on younger animals of the chloride values given in table 1. In this second experiment, additional data concerning blood volume and blood glucose changes have also been recorded.

As the tables indicate, the whole blood chlorides are decreased after adrenalectomy and restored towards or to normal by D.C.A. but they

remain unaffected by progesterone. The plasma chloride values run approximately parallel to the whole blood chlorides except in the adult females (table 1) in which the average plasma chloride concentration was not below normal either in the case of D.C.A. or adrenalectomy. This might perhaps be ascribed to the fact that the intact controls of this particular group showed an unusually low normal whole blood and plasma chloride concentration for females. As we pointed out in a previous publication (2) usually the normal blood chlorides of females are significantly higher than those of males. In any case the plasma chloride variations, though essentially similar to those of the whole blood, were always much less pronounced. This is in agreement with the view that the hypochloremia produced by adrenalectomy or D.C.A. is mainly due to a decrease in red cell chlorides (2, 6). The muscle chlorides are only insignificantly increased by D.C.A. or adrenalectomy but show a marked rise in D.C.A.-treated adrenalectomized animals. Progesterone has no such effect. No consistent change has been observed in the chloride concentration of the liver or brain in any of these groups, hence we omitted the corresponding values from our tables. The average circulating blood volume was not significantly changed by D.C.A. in the intact rats of this series because one animal had an unusually low blood volume. However, numerous other experiments lead us to believe that there is a constant increase in the blood volume of normal rats treated with large doses of D.C.A. Thus in a group of four male and four female rats weighing 165 grams on the average, the blood volume was 3.5 ml. while in a similar group of rats treated with 2 mgm. of D.C.A. daily during 47 days, it was 3.9 ml. ($P = <0.01$). The hematocrit and hemoglobin values showed no significant change, however, so that the condition appears to be one of true plethora. Following adrenalectomy, we observed the usual decrease in blood volume. This could be restored by D.C.A. but not by progesterone.

The blood glucose showed a marked decrease in D.C.A.-treated intact animals while in adrenalectomized rats, D.C.A. tended to restore the low blood sugar towards normal. Progesterone showed no such effect. It should be emphasized, however, that in other experiments D.C.A. failed to cause consistent hypoglycemia. It is noteworthy that progesterone proved entirely inactive in preventing the hypoglycemia of the adrenalectomized animals.

It is evident from the experiments reported above that the same dose of D.C.A. which decreases the blood chlorides in the presence of the adrenals not only fails to do so in the adrenalectomized animal but actually exhibits an inverse action inasmuch as it restores the otherwise low blood chlorides of the adrenal-deficient rat to normal. Tissue chloride determinations failed to throw more light on this curious response since the liver and brain showed no consistent change in chloride concentration under any of the

experimental conditions investigated here. The muscle chlorides revealed only an insignificant rise in D.C.A.-treated intact animals and a much more pronounced rise in adrenalectomized animals in which the blood chlorides were also raised by this compound. Preliminary studies concerning the chloride excretion of D.C.A.-treated rats indicate, however, that concomitantly with the decrease in blood chlorides, the urinary chloride elimination is likewise markedly increased in all cases. This is due partly to a slight and inconsistent rise in the chloride concentration of the urine but mainly to a considerable increase in the total urine output (7).

In order to elucidate the rôle played by the adrenals in influencing the action of D.C.A. on the blood chlorides, another series of experiments was performed on 12 male rats weighing 63 to 85 grams (average 75 grams). Six of these were pretreated with 2.5 mgm. of D.C.A. given twice daily subcutaneously in 0.1 ml. of peanut oil while six controls received only

TABLE 3

Blood chlorides in rats chronically treated with D.C.A.

DAYS	TREATMENT	WHOLE BLOOD CHLORIDES	TREATMENT	WHOLE BLOOD CHLORIDES
0	Oil	413 (392–433)	D.C.A.	422 (398–445)
8		416 (398–456)		353 (346–369)
9	Adrenalectomy		Adrenalectomy	
10	D.C.A.	420 (363–456)	D.C.A.	383 (363–398)
15		395 (374–416)		352 (316–392)
20		333 (316–363)		335 (304–357)

an equivalent amount of peanut oil during the first 8 days of the experiment. On the ninth day, both groups were adrenalectomized. Treatment with D.C.A. was continued in the D.C.A. pretreated group but immediately after the operation the not pretreated controls also received D.C.A. in the same dosage. On the day before adrenalectomy, the blood chlorides in the D.C.A. pretreated group had already decreased considerably below their initial value while those of the not pretreated controls did not change. After adrenalectomy the animals having received D.C.A. only after the operation showed essentially normal blood chloride values on the day following the operation, the D.C.A. having prevented the usual decrease in blood chlorides. In the hypochloremic D.C.A. pretreated group the chlorides actually rose after the operation though not quite to the normal level. These observations are in accord with previously reported findings indicating that adrenalectomy prevents the hypochloremic action of D.C.A.; in fact, they show that the hypochloremia, if already established as a result of D.C.A. pretreatment, tends to disappear

after ablation of these glands. However further continuation of the same treatment indicated that later the blood chlorides decrease to exceedingly low values in both groups even though subsequent autopsy showed no trace of an adrenal remnant. Table 3 summarizes these results.

It is difficult to understand why adrenalectomy temporarily prevents the hypochloremic action of D.C.A. However the known facts are compatible with the assumption that this action of the compound is not a direct one but is mediated by some other organ which in turn is influenced by the adrenals and requires some time after adrenalectomy to recuperate its responsiveness to D.C.A. In view of the fact (7) that after hypophysectomy rats permanently lose their ability to respond to D.C.A. with a decrease in blood chlorides, it is tempting to assume that this action of the hormone is mediated by the hypophysis.

SUMMARY AND CONCLUSIONS

In the intact rat, D.C.A. decreases the whole blood chlorides while in adrenalectomized animals whose blood chlorides are subnormal, the same dose of D.C.A. temporarily raises them to or towards normal. Prolonged D.C.A. administration on the other hand decreases the blood chlorides even after adrenalectomy. The plasma chlorides run roughly parallel with the whole blood chlorides in most cases but their variations are less pronounced than those of the latter. From this it may be concluded that the hypochloremia caused by adrenalectomy or by administration of D.C.A. in normal rats is due mainly to a decrease in red cell chlorides.

As a rule the muscle chlorides are only insignificantly increased by D.C.A. or adrenalectomy but always show a pronounced increase in D.C.A.-treated adrenalectomized animals. The brain and liver chlorides reveal no consistent change after adrenalectomy or after the administration of D.C.A. to intact or adrenalectomized animals.

The blood volume which is decreased by adrenalectomy is restored by D.C.A. administration and in the intact rat D.C.A. causes a slight rise in blood volume.

The blood glucose concentration is decreased by adrenalectomy and restored towards normal, though usually not to normal, by D.C.A.

Progesterone given in doses as high as 10 mgm. per day has no effect on the low blood volume, blood sugar and blood chlorides of adrenalectomized rats in acute experiments. This is of interest because in the case of chronic treatment with this compound other investigators reported progesterone to have essentially the same life-maintaining effect in adrenalectomized rats as D.C.A. has. Since most of the other known physiological actions of these two compounds (progestational proliferation of endometrium, mammary gland stimulation, etc.) are likewise identical, experiments such

as those reported in this communication appear to be especially suitable for pharmacological differentiation between these two compounds. These observations also demonstrate the importance of the —OH group on C-atom 21 for the exertion of the pharmacological actions of these compounds since the substitution of this group by hydrogen is the only chemical difference between the molecules of D.C.A. and progesterone.

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GUM ACACIA AND THE OXYGENATION OF RED CELLS

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The following five groups of experiments are presented as evidence that gum acacia, in concentrations common to clinical usage, neither inhibits nor retards the rate of oxygenation of red cells in vitro or in vivo. The conclusions drawn from these experiments differ from those of Christie, Phatak and Olney (1), who state that gum acacia, when present in the blood stream, retards the rate of oxygen transport across the plasma membrane of the red cells.

METHODS. The first, fourth and fifth groups of experiments were designed particularly for the present study. The second and third groups were obtained from animals subjected to total plasmapheresis with a gum acacia-red cell-Locke's solution. The data which were obtained from the analysis of the various blood-gum acacia mixtures are recorded in terms of a ratio in which the volumes per cent of oxygen actually found in a test sample is related to the total oxygen capacity of the sample as determined by an analysis of the total hemoglobin. The actual oxygen content of each blood sample was determined by the manometric method of Van Slyke (2). The total hemoglobin content (oxygenated-, reduced-, and met-hemoglobin) was measured as cyanmethemoglobin according to the method of Drabkin and Austin (3). Through the use of proper conversion factors, the value for total hemoglobin so obtained was expressed in terms of its equivalent volumes per cent of oxygen. Blood samples were collected anaerobically by a method described elsewhere (4).

Many of the blood samples may have contained small amounts of methemoglobin, since conditions for its slow formation were inherent in some of the procedures. Since the effect being investigated had been stated to be a large one (1), no attempt was made to overcome the smaller sources of error. The oxygen saturation of hemoglobin within the lungs is about 94 to 96 per cent complete. Oxygen saturation values of 90 to 94 per cent in our data may be considered to be within the range normally observed.

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OBSERVATIONS. 1. *Red cell-gum acacia mixtures in vitro.* Normal red cells were washed three times in a buffered Locke's solution and, after washing, were deoxygenated in an evacuated flask. Five cubic centimeters of cells were then suspended in an equal volume of each of the various solutions listed in table 1, placed in a rotating tonometer, and oxygenated in a stream of moist air. No attempt was made to obtain the best possible samples of cells since the data were of comparative value only. No evidence was found that the presence of gum acacia in any way influenced the oxygenation of red cells under these conditions (table 1).

2. *Resuspended venous "acacia cells."* In one stage of the experimental work, we obtained small samples of red cells from dogs which had undergone total plasmapheresis. These cells, centrifuged free of plasma, were

TABLE 1

	1	2	3	4	5
	cc. of cells				
	5	5	5	5	5
Suspension solution	5 cc. of serum	2.5 cc. of serum and 2.5 cc. 6 per cent gum acacia in saline	5 cc. of 6 per cent gum acacia in saline	5 cc. of 6 per cent gum acacia in Locke's solution	5 cc. of 6 per cent gum acacia in Ringer's solution
Oxygen content / Oxygen capacity	0.926	0.934	0.936	0.942	0.945

resuspended in a volume of Locke's solution equal to that of the withdrawn plasma, placed in a rotating tonometer, and oxygenated for a period of fifteen minutes in a stream of moist air. The two experiments recorded in table 2 are from a series of six such experiments. In every case, the cells were oxygenated to values well within the normal limits. The low value of the second figure in column 6 in the table is related to an extensive hemolysis in the test sample, and is attributable to the presence of methemoglobin which forms readily when free hemoglobin is present within the blood stream (5).

3. *Oxygen content and oxygen capacity of "gum acacia" venous blood.* Venous blood was withdrawn anaerobically from dogs which had undergone plasmapheresis with a gum acacia-red cell Locke's solution. One portion of the sample was immediately analysed for its oxygen content, while another portion was first saturated with the oxygen of room air in a rotating tonometer and then analyzed. Each value was then related, in

terms of the ratio previously described, to the volumes per cent of oxygen represented by the total amount of hemoglobin present in the sample. The data of table 3 are from one of five such experiments.

The oxygen content of the venous blood obtained just after the operation was very high. This, we believe, was due to the lowered oxygen consumption of the animal during the period of anesthesia under which the

TABLE 2

ANIMAL	TIME	RESUSPENDED "VENOUS CELLS"		
		Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5
P	Before	21.9	21.0	1.042
	After	17.0	19.3	0.883
	2:45	20.6	21.2	0.973
	15:00	20.4	20.8	0.982
	27:30	20.8	20.8	1.000
R	Before	17.3	17.5	0.988
	After	16.0	15.6	1.030
	0:48	13.9	13.2	1.050
	3:15	14.2	14.0	1.010

TABLE 3

ANIMAL	TIME	HEMATOCRIT READ IN PER CENT	VENOUS BLOOD			OXYGENATED VENOUS BLOOD		
			Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity	Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5	6	7	8	9
W	Before	59	6.91	17.51	0.394	16.74	17.51	0.934
	0:00	56	14.40	19.38	0.744	19.02	19.38	0.947
	1:55		7.09	19.78	0.458	19.90	19.78	1.010
	4:00	55	6.65	19.50	0.431	19.35	19.50	0.990
	6:05	52	6.29	16.00	0.392	17.00	16.00	1.032
	15:30	40	5.76	12.68	0.455	13.00	12.68	1.025

operation was performed (6). Later values were within the normal range. If the gum acacia acts to inhibit the passage of oxygen across the plasma membrane of the red cell (1), one would expect that the volumes per cent of oxygen contained in the venous blood would be higher than normal. This would be a direct result of the statement that the cells did not unload the oxygen due to a restricting surface film of gum acacia. We found no evidence of such restricted movement of oxygen (column 4). As can be

seen in column 7 of table 3, the in vitro oxygenation of the venous blood also resulted in normal values, even though the cells had been in the presence of gum acacia within the blood stream for quite long periods.

4. *Oxygen content of arterial and venous blood in anesthetized dogs.* Normal dogs were anesthetized with dial (60 mgm./kgm.), and branch vessels of both the femoral vein and artery were cannulated in such a manner that the tips of the cannulae extended into the flowing blood stream of primary vessels. Thirty per cent gum acacia in 4.5 per cent saline solution was slowly injected intravenously in an amount such that the gum acacia concentration totalled 1.6 grams per cent of the estimated plasma volume. The deep anesthesia caused by the dial was accompanied with marked respiratory depression which in turn resulted in a rather low degree of oxygen saturation of the arterial blood (column 8, table 4). The data

TABLE 4

ANIMAL	TIME	VENOUS			ARTERIAL		
		Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity	Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5	6	7	8
	hours						
9-M	Before	8.68	22.50	0.386	20.16	22.50	0.905
Injected 40 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 1.30 per cent							
Dial: 60 mgm. per kgm.	0:15	8.38	21.56	0.389	18.84	21.65	0.872
	2:15	8.06	22.08	0.366	19.56	21.00	0.932
	4:40	9.20	25.80	0.356	21.62	24.92	0.872

shown in table 4 give no evidence that the injection of gum acacia affected the normal relationship between the oxygen content of the venous and that of the arterial blood.

5. *Oxygen content of arterial and venous blood in unanesthetized dogs.* In a final group of five experiments, performed on normal unanesthetized dogs, 30 per cent gum acacia dissolved in 4.5 per cent saline solution was slowly injected intravenously in quantities sufficient to produce a plasma concentration of about 3 grams per cent. Venous blood from the jugular vein and arterial blood from the femoral artery was collected anaerobically at various time intervals after the injection, and was analyzed for its oxygen content and its total hemoglobin. Since this group of experiments is the most decisive in the entire series, two examples are presented in table 5 as evidence that gum acacia produces no demonstrable effect on the oxygen carrying capacity of the red cells.

Discussion. These data indicate that in a variety of conditions, both in vitro and in vivo, gum acacia has no inhibiting or retarding effect upon

the oxygenation of red cells. The small variations, and the occasional larger deviations in the data from a normal level of arterial and venous oxygen values are adequately and satisfactorily explained in terms of the physiological conditions of the moment. Christie, Phatak and Olney (1) have referred only briefly to the clinical signs that led them to suspect deficient gas transport in their patients. It is difficult to evaluate their observations. Studdiford (7), however, describes three instances of severe reactions following the injection of gum acacia into postpartum patients, and describes clinical signs of cyanosis and coma. These, he suggests, may be related to the experimental observations of Christie, Phatak and Olney (1). In view of the findings described in our experiments, we are

TABLE 5

ANIMAL	TIME	VENOUS			ARTERIAL		
		Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity	Vol. per cent oxygen	Total Hb	Oxygen content / Oxygen capacity
1	2	3	4	5	6	7	8
	hours						
10-B	Before	7.65	14.60	0.523	13.72	14.31	0.960
Injected 40 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 2.96 per cent							
	0:55	9.10	9.78	0.888	11.54	12.03	0.960
	16:30	7.52	12.42	0.605	13.30	13.62	0.974
	42:00	7.30	14.64	0.512	13.72	14.72	0.932
10-E	Before	14.42	21.60	0.675	18.80	20.15	0.935
Injected 35 cc. of 30 per cent gum acacia in 4.5 per cent NaCl solution to give a calculated plasma concentration of 2.92 per cent							
	0:33	10.54	19.00	0.555	17.00	18.60	0.915
	4:30	12.20	19.30	0.658	17.35	18.15	0.968
	16:50				17.72	18.15	0.952
	43:50				16.85	18.00	0.940

entirely skeptical of the usefulness of data secured after such massive injections of gum acacia as were given by the latter authors. Such injections cause an embarrassing increase in blood volume and probably blood viscosity. We may conclude that the adverse reactions which they describe are most likely explicable in terms of unknown factors peculiar to their study. Amberson (8), in a review of blood substitutes, mentions instances during the first World War, when, in soldiers who had undergone a very severe hemorrhage, gum acacia solutions were ineffectual in sustaining life. He suggested, on the basis of the observations reported by Christie, Phatak and Olney (1), that the injections of acacia into such patients actually may have made their critical condition worse by placing a handicap on their red cells which were already insufficient in number.

At the moment, however, it is safe to conclude that, in each of these instances, the basic cause of death was solely a reduction of the circulating red cells to a level insufficient for the maintenance of life.

Second to whole blood, or lyophilized plasma, gum acacia remains the most satisfactory blood adjuvant available and should be indicated as an emergency measure whenever blood or plasma are not available. Increasing caution and consideration are to be observed, however, when the problem of repeated injections presents itself. The work of Andersch and Gibson (9), and more recently that of Heckel, Erickson, Yuile and Knutti (10), has established the fact that complications beyond control result from multiple injections of gum acacia. A certain amount of gum acacia given in each injection is taken up by the liver and stored as an inert material within the hepatic cells. When repeated injections are given, such large amounts of the gum are eventually stored in the liver that drastic disruption of the normal fun ning of the organ is brought about.

SUMMARY

Data have been presented indicating that g acacia, when injected into the blood stream, does not retard, or in any v affect, the movement of oxygen across the plasma membrane of the red ll. This is in contradiction to observations made and recorded by ot r authors. Experiments in vitro, as well as experiments on both arter l and venous blood, before and after the addition of acacia, have in no i tance shown variations in oxygen saturation of the red cells beyond ose found in the controls.

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THE RELATION BETWEEN THE FAT AND CARBOHYDRATE METABOLISM OF LACTATION, AS INDICATED BY THE RESPIRATORY QUOTIENT OF THE MAMMARY GLAND

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Although the individual milk precursors withdrawn from the blood stream by the lactating mammary gland have been the subject of numerous investigations, the respiratory quotient of the gland has not received attention until quite recently.

Graham, Houchin, Peterson and Turner (1) reported that the respiratory quotient of the active mammary gland is considerably above unity, suggesting the synthesis of a portion of the milk fat from carbohydrate. Shaw (2) reported that respiratory quotients of the intact mammary gland were difficult to evaluate because of the great variations encountered. However, he stated that the respiratory quotient of the excised perfused gland varied but little from the value of 0.80. This fact together with the claim of Shaw and Petersen (3) that an excess of fat in proportion to other milk precursors is taken up by the active mammary gland was believed to indicate the partial oxidation of fat in the gland. It was postulated that this would account for the lower fatty acids of milk fat.

In view of this rather marked divergence of results, the present experiments were undertaken with the object of establishing more definitely the respiratory quotient of the normal intact gland, as well as some of the factors that will affect this value.

METHODS. Blood samples were drawn simultaneously from the "exteriorized" carotid artery and mammary vein of normal, intact goats. A series of samples obtained without anesthesia and a second series secured following anesthetization of the blood vessels with apothesine were drawn with the animal standing quietly in its stall. However, a certain amount of excitement could not be avoided in about 50 per cent of the cases. Further experiments (unpublished) showed that goats under nembutal anesthesia continue to secrete milk of normal composition at an undiminished rate, and that the uptake of milk precursors by the mammary gland continues at the normal level. Accordingly, large series of samples were drawn from nembutalized animals. The blood samples were collected

under oil into flasks coated with equal amounts of potassium oxalate. They were chilled at once by immersion in an ice water bath, and immediately taken to the laboratory for analysis. Oxygen and carbon dioxide were determined by the manometric method of Van Slyke and Neill (4) and hemoglobin by the method of Evelyn and Malloy (5).

RESULTS. The effect of the various methods of sampling upon the level of oxygen and carbon dioxide in the blood, the respiratory quotient, and the hemoglobin difference is shown in condensed form in table 1. In the

TABLE 1

Showing analyses of arterial and mammary bloods, with calculated respiratory quotients of the lactating mammary gland

NO. OF EXPERIMENTS	MEAN OXYGEN			MEAN CARBON DIOXIDE			MEAN R.Q.	HEMOGLOBIN PER CENT DIFFERENCE
	Arterial	Mammary	Difference	Arterial	Mammary	Difference		
Series I. Blood samples drawn with no preliminary anesthesia								
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
20	8.49	4.43	+4.07	49.32	53.26	−4.79	1.17 ±0.0361	0.57 (8 values)
Series II. Blood samples drawn with blood vessels anesthetized locally								
15	8.44	4.38	+4.07	50.31	55.03	−4.72	1.15 ±0.034	2.59 (14 values)
Series III. Blood samples drawn under nembutal anesthesia; animals quiet, respiration unimpeded								
29	9.88	5.81	+4.07	53.41	57.84	−4.435	1.09 ±0.0115	1.08 (25 values)
Series IV. Nembutal anesthesia excessive; respiration impeded								
7	9.22	5.16	+4.03	64.06	68.34	−3.21	0.81	3.24 (5 values)

experiments conducted without anesthesia, the respiratory quotient varied between 0.86 and 1.84, with a mean value of 1.17 ± 0.0361. The hemoglobin values obtained in this series showed no significant changes in concentration of the two types of blood. The use of a local anesthetic about the blood vessels (series II) had little effect in reducing the variability of the carbon dioxide:oxygen ratio as shown by the range of 0.92 to 1.68, and mean respiratory quotient of 1.15 ± 0.034. Series III includes the results of 29 experiments in which blood samples were drawn from goats under nembutal anesthesia, with no respiratory complications. When compared to the preceding trials it will be noted that the respiratory quotient is quite constant, varying but little from the mean of 1.09.

It is of interest to note that the average uptake of oxygen by the mammary gland was identical in each of the first 3 series. Thus any differences in the average respiratory quotient are due to differences in the carbon dioxide exchange. Furthermore, it seems reasonable to suppose that one of the immediate effects of excitation of the animal would be an increased rate of ventilation of the lungs that would cause abnormal expiration of carbon dioxide. This in turn would promote a greater transfer of carbon dioxide from the tissues to the venous blood, resulting in a higher carbon dioxide:oxygen ratio. Anesthetization of the animal before drawing blood samples eliminates this effect and results in much more uniform values. Further support for this concept is found in series IV, containing the results of experiments in which the anesthesia was excessive, and respiratory difficulties were encountered as a result. Because of the faulty respiration in these cases there was an abnormally high carbon dioxide level in the blood, which in turn would lead to accumulation of carbon dioxide in the tissues. Thus the low respiratory quotients recorded are more apparent than real.

The fact having been established that the respiratory quotient of the mammary gland of the lactating goat varies within narrow limits about the mean of 1.09, it seemed of interest to obtain some information on mammary gland metabolism in other physiological states. Results obtained on non-lactating goats during the last half of pregnancy, in addition to data on one non-lactating-non-pregnant goat and three lactating-fasted goats are presented in table 2.

The rather striking fact comes to light that the respiratory quotient of the mammary gland of the non-lactating-pregnant goat (series V) is identical with that of the goat in normal lactation. This agrees with the evidence that mammary development is complete and milk secretion begins about mid-pregnancy.

Unfortunately, only one non-lactating-non-pregnant goat was available for sampling, while this investigation was in progress (series VI). However, all of the ratios fall between the values of 0.68 and 0.98, with the average of 0.81. It appears, therefore, that the metabolic needs of the inactive gland may be met by the combustion of either fat or carbohydrate, or both, while the active gland normally uses some carbohydrate for fat synthesis. Another rather interesting point revealed in series V and VI is that the uptake of oxygen by the mammary gland of non-lactating goats is practically the same per unit flow of blood as for normally lactating goats. Therefore, the increased metabolic requirements of the gland during lactation must be met by a marked increase in the rate of blood flow.

In view of the constancy of the respiratory quotient of the mammary gland during lactation, under the given conditions, it seemed of interest to determine whether or not this ratio would be maintained during fasting, when the carbohydrate stores of the organism would be seriously depleted.

The results of such experiments with three goats are included in series VI. Goats 836 and 444 were in good condition at the start of the fast and their respiratory quotients remained above unity for the first 48 hours,

TABLE 2

Showing analyses of arterial and mammary bloods, with calculated respiratory quotient of nonlactating-pregnant, nonlactating-nonpregnant and lactating fasted goats

GOAT NO.	OXYGEN			CARBON DIOXIDE			R.Q.	HEMOGLOBIN PER CENT DIFFERENCE	DURATION OF FAST
	Arterial	Mammary	Difference	Arterial	Mammary	Difference			
	Series V. Nonlactating pregnant goats								
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*			*hours*
1	9.41	6.52	+2.89	51.09	54.23	−3.14	1.09	−1.13	
2	10.88	6.91	+3.97	46.35	50.64	−4.29	1.07		
3	10.56	5.97	+4.59	50.25	55.22	−4.97	1.08	+0.64	
3	11.09	5.56	+5.43	46.77	52.64	−5.87	1.08	+1.17	
3	9.12	6.01	+3.11	49.88	53.22	−3.34	1.08	+0.24	
3	9.94	5.56	+4.38	41.66	46.38	−4.72	1.08	−2.66	
3	10.08	6.04	+4.04	46.67	51.02	−4.35	1.08	+0.52	
3	8.82	5.54	+3.28	45.74	49.56	−3.82	1.17	+0.06	
3	9.96	5.32	+4.64	49.42	54.29	−4.87	1.05	+0.67	
Mean..	9.98	5.94	+4.04	47.53	51.91	−4.37	1.09	0.88	
	Series VI. Nonlactating-nonpregnant goat								
4	10.18	5.82	+4.36	54.49	57.21	−2.72	0.62	+0.48	
4	11.31	4.52	+6.79	49.87	56.56	−6.69	0.98	−1.41	
4	10.13	4.35	+5.78	46.45	51.59	−5.14	0.89	−1.21	
4	9.68	5.40	+4.28	53.20	56.39	−3.19	0.75	−0.84	
4	8.38	5.25	+3,13	60.55	63.12	−2.57	0.85	+0.53	
4	10.75	5.32	+5.43	56.17	60.37	−4.20	0.77	−0.27	
Mean..	10.07	5.11	+4.96	53.45	57.54	−4.08	0.81	0.79	
	Series VII. Lactating fasted goats								
5	12.11	8.99	+3.12	51.69	54.93	−3.24	1.04	+0.09	40
6	7.77	6.74	+1.03	56.08	57.30	−1.22	1.18	−3.11	48
7	6.33	4.12	+2.21	52.14	54.05	−1.91	0.87		42
7	9.27	3.69	+5.58	44.68	49.92	−5.24	0.94		66
5	13.67	9.11	+4.56	51.71	55.47	−3.78	0.83		64
6	7.44	5.65	+1.79	57.75	58.97	−1.22	0.68		72
Mean..	9.43	6.38	+3.05	52.34	55.10	−2.76			

followed by a drop to 0.87 and 0.68, respectively, on the following day. Goat 434 was poorer in body flesh at the start of the fast and her mammary respiratory quotient had declined below unity by the 42nd hour. It

appears likely, therefore, that fat synthesis in the mammary gland continues only as long as ample stores of carbohydrate are available.

DISCUSSION. In considering the respiratory quotient of the active mammary gland of the goat, presented in table 1, series I, II and III, no support can be found for the claim that fats are partially oxidized and reduced, thus accounting for the lower fatty acids of milk. On the contrary, the finding of Graham, Houchin, Peterson and Turner (1) that the respiratory quotient of the lactating mammary gland is above unity, indicating the synthesis of a portion of the milk fat from carbohydrate, has been confirmed and extended. It appears probable that the average value of 1.09 obtained in series III, where the effects of excitement had been eliminated by the use of nembutal anesthesia, very nearly approximates the true oxygen and carbon dioxide exchange in the normal mammary gland. Graham et al. corrected their data for the amount of urea synthesized in the mammary gland, estimating that the average urea output would account for 0.392 vol. per cent of added carbon dioxide. If we assume that a similar output of urea occurred in the present experiments and add 0.392 vol. per cent of carbon dioxide to the values in table 1, series III, we arrive at a corrected respiratory quotient of 1.18. In either event the respiratory quotient is sufficiently high, in view of the constancy of the results, to indicate a mammary gland metabolism favoring some fat synthesis from carbohydrate.

It is of interest to correlate the decline in respiratory quotient of the mammary gland during fasting with the changes that are known to take place in the fatty acids of the milk fat during a similar period. The pioneer investigations of Eckles and Palmer (6, 7) showed that the plane of nutrition has a marked influence on the character of the milk fat. It was shown that all types of underfeeding cause a decline in the Reichert-Meissl and saponification numbers and an increase in the iodine number, while overfeeding and feeding at the normal plane of nutrition maintained these constants at their normal value. More recently it was reported by Smith and Dastur (8) that in milk fat produced during a prolonged fast there was a decrease of about 80 per cent in the original content of the lower fatty acids, up to and including C_{14}, a deficiency that was almost entirely made good by an increase in oleic acid. In view of the parallel decline of the respiratory quotient and the lower fatty acids of milk fat it appears extremely likely that at least a portion of these acids are normally synthesized from carbohydrate. However, it is also well established that neutral fat derived from the blood stream is available for the needs of lactation. Thus it appears that the milk fat must be derived from at least two different types of precursors:

1. The higher fatty acids are probably derived from the neutral fat of the blood, as shown by the uptake of neutral fat.

2. The lower fatty acids are formed, at least in part, by synthesis from carbohydrate. The derivation of a portion of these acids from fats of low molecular weight or fat-like fragments that could be taken up from the blood stream in small amounts is not excluded, however.

A further point that may have some significance is the fact that the respiratory quotient of the mammary gland of fasted goats is very similar to the value of 0.80 reported by Shaw (2) and Petersen, Shaw and Visscher (9) for the perfused excised udder. Unless enough is known about the requirements for normal milk secretion to enable the addition of the proper amounts of milk precursor, hormones, etc., the blood passing through the perfusion system would very shortly present a picture of severe inanition insofar as the mammary gland is concerned.

The concept of the synthesis of a portion of the milk fat from carbohydrate implies a further drain on the heavily taxed carbohydrate metabolism of the mammary gland in addition to that needed for lactose synthesis and tissue maintenance. The carbohydrate balances reported by Graham (10) and the ratios of Shaw (2) indicate that the uptake of glucose and lactic acid will account only for the synthesis of lactose. Further experiments from our laboratory (11) indicate that additional carbohydrate, to the extent of about 2.0 mgm. per 100 ml. is taken up by the mammary gland from the blood plasma as a portion of a glycoprotein complex. Since the milk proteins contain only traces of sugar (12), it appears probable that the greater portion of this moiety will be available for use as carbohydrate.

SUMMARY AND CONCLUSIONS

1. The average respiratory quotient of the active mammary gland in 20 experiments on normal unanesthetized goats was 1.17 $\pm$ 0.0361. Local anesthetization of the blood vessel sampled in 15 experiments resulted in a mean respiratory quotient of 1.15 $\pm$ 0.034. Use of nembutal as a general anesthetic during the sampling of blood greatly reduced the variability of the values, resulting in a mean respiratory quotient of the active mammary gland of 1.09 $\pm$ 0.0115. Correction for the synthesis of urea in the mammary gland would increase this figure to approximately 1.18.

2. The average uptake of oxygen by the mammary gland was identical in the three series of experiments noted above. It was suggested, therefore, that the chief cause of variation in the apparent respiratory quotient in unanesthetized animals was due to variations in the rate of ventilation of the lungs, which would cause variations in the amount of carbon dioxide expired.

3. The respiratory quotient of the mammary gland of the non-lactating-pregnant goat during the last half of pregnancy was found to be identical with that of the lactating goat, while that of a non-lactating-non-pregnant goat had declined to 0.81.

4. The mammary gland of goats fasted for periods up to 72 hours and sampled under nembutal anesthesia showed a decline in the respiratory quotient to below unity by the third day.

5. Because of the parallel between the decline of the respiratory quotient of the mammary gland and the known decrease in the lower fatty acids of milk fat during fasting, it is suggested that the synthesis of milk fat from carbohydrate is confined largely to the fatty acids of low molecular weight.

6. The relationship between the synthesis of milk fat from carbohydrate and the carbohydrate balance of the lactating mammary gland is discussed.

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THE RESPONSE OF THE DENERVATED NICTITATING MEMBRANE AND OF BLOOD PRESSURE TO SYMPATHETIC NERVE STIMULATION IN ADRENALECTOMIZED CATS

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In cats moribund from adrenal insufficiency, stimulation of sympathetic vasomotor nerves causes little or no rise in blood pressure. The injection of a variety of pressor drugs, with the exception of epinephrine, is similarly ineffective (Elliott, 1904, 1914; Cleghorn, 1938, 1939; Armstrong, Cleghorn, Fowler and McVicar, 1939). In order to explain this phenomenon, the theory that an adrenal cortical hormone might be concerned in some way with the elaboration of sympathin has been considered. Evidence in favour of such a theory has been advanced by Secker (1937, 1938). He found that the response to repeated stimulation of cervical and mesenteric nerves failed much more rapidly in adrenalectomized cats than in normal animals. In view of this observation, it might be thought that adrenalectomized animals, maintained without cortical hormone, would show impaired responses to sympathetic nerve stimulation. This hypothesis has been tested in adrenalectomized dogs maintained for many weeks without cortin by using a diet high in salt and low in potassium. The rise in blood pressure resulting from splanchnic nerve stimulation in these animals was quite as good as in controls (Cleghorn, Armstrong and Austen, 1938). Further evidence against the position of Secker lies in the finding that cats in severe adrenal insufficiency respond to stimulation of the cervical sympathetic by a strong and sustained contraction of the innervated nictitating membrane (Armstrong et al., 1939). In this report it was suggested that the apparent paralysis of cardioaccelerator and vasoconstrictor nerves in such animals might be due to exhaustion of sympathin at the endings of those nerves. It was felt that sustained hyperactivity of vasomotor pressor mechanisms might result from the diminished blood volume and lead to this state of exhaustion. An account of our efforts to compare quantitatively the liberation of sympathin at vasomotor nerve endings in healthy cats and also in cats in various stages of adrenal insufficiency is presented here.

METHODS. Care of the animals, the method of adrenalectomy, chemical

and other techniques were carried out as described by Armstrong, Cleghorn, Fowler and McVicar (1939). The denervated right n.m.[1] was utilized as the peripheral indicator of sympathin, as described by Rosen-

TABLE 1

Healthy adrenalectomized cats: The effect of epinephrine injections and of sympathin on the contraction of the denervated nictitating membrane

CAT NUMBER	HEIGHT OF RECORDED CONTRACTION OF N.M. IN RESPONSE TO:		
	Epinephrine injections*		Sympathin R. Spl. Stim.†
	0.1γ	1.0γ	
Series A. Cortin-treated			
	mm.	*mm.*	*mm.*
1	1	21	15
2	2	25	18
3	11	62	70
4	4	77	88
5	9	44	40
Average	5	46	46
Series B. Acutely adrenalectomized			
6	24		45
7	9		49
8	20		51
9	26	58	69
10	18	31	62
11		33	30
12		30	58
13		38	34
14		43	38
Average	19	39	48

Series A: Cats adrenalectomized 21 to 41 days prior to experiment and maintained by cortin.

Series B: Cats adrenalectomized immediately before experiments.

* Epinephrine injected directly into a femoral vein over a period of 10 seconds in a volume of 1 cc.

† Splanchnic nerve isolated and cut below diaphragm; stimulated supramaximally for 30 seconds; blood pressure rise resulting averaged 44 mm. Hg for series A and 34 mm. Hg for series B.

Contractions of n.m. recorded isotonically; tension 4 grams; magnification ×15.

blueth and Cannon (1932). The n.m. was denervated by excision of the right superior cervical ganglion at least three weeks prior to the experiments in order that maximal sensitivity might be developed (Hampel,

[1] N.m., nictitating membrane.

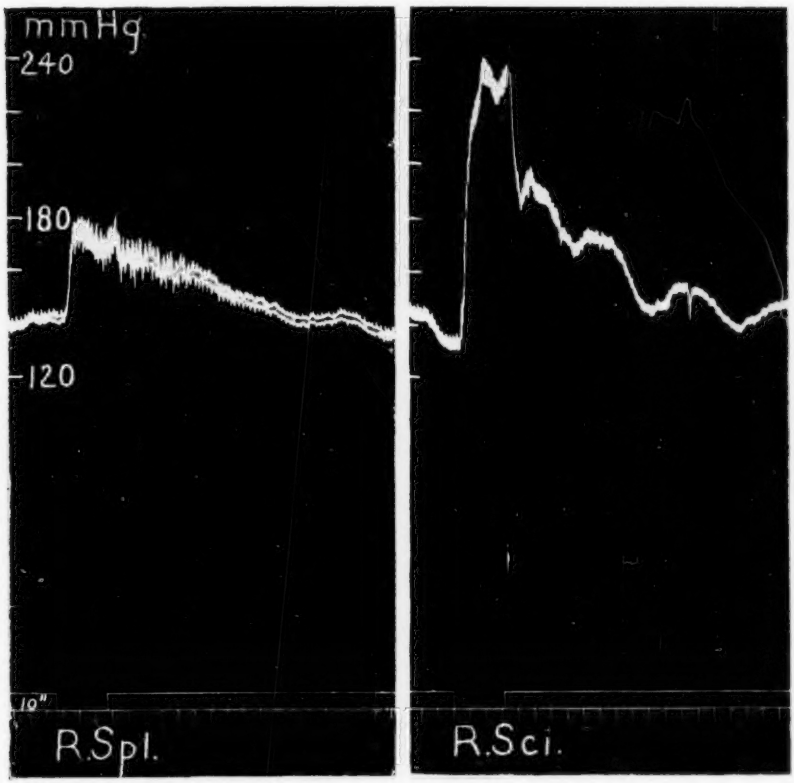
1934). It has been found that this sensitivity persists for a minimum of ten weeks. In the animals in which sympathin liberation was studied some days or weeks after adrenalectomy, the ganglion and right adrenal were removed at one operation. The second gland was excised a week or two later. Urethane was used as the anesthetic (Liu and Rosenblueth, 1935) since it permits liberation of sympathin by reflex as well as by direct stimulation of efferent nerves. In the experiments on animals in adrenal insufficiency, chemical changes in the blood, characteristic of this state, were found. At autopsy no residual cortical tissue was seen in cortin-treated cats nor in those allowed to develop adrenal insufficiency.

RESULTS. 1. *Examination of Cortin-treated, Adrenalectomized Cats.* (a) *Effect of brief stimulation of sympathetic nerves.* The n.m. response to splanchnic nerve stimulation appeared to be normal in cortin-treated cats compared to acutely adrenalectomized controls (table 1). It was also observed that stimulation of a splanchnic nerve for 30 seconds elicited a contraction of the n.m. similar in magnitude to that produced by 1.0γ of epinephrine injected intravenously over a period of 10 seconds. This quantity of epinephrine produces a submaximal response of the n.m. This finding supplied a means of assaying the amount of sympathin liberated into the blood stream. The experiments listed in table 1 also demonstrate that the comparison of the sympathin liberated by splanchnic stimulation and the standard 1.0γ dose of epinephrine must be made on the same animal. The n.m. contractions following splanchnic stimulation in cats 10 and 12 (series B) were exceptionally large compared to the response to epinephrine. There appeared to be no constant ratio between the effect elicited by 0.1γ and 1.0γ of epinephrine. Central stimulation of a cut sciatic nerve gave a pressor response about twice that seen with splanchnic stimulation, but the n.m. response was not proportionately greater. Sympathin liberation in a cortin-treated cat is illustrated in figure 1 a and b.

(b) *Effect of prolonged stimulation of sympathetic nerves.* Prolonged splanchnic nerve stimulation was carried out in seven cats which had been maintained 13 to 39 days after adrenalectomy by cortin. The blood pressure remained elevated above prestimulation level for an average of 24 minutes, with a range of 15 to 35 minutes. In seventeen acutely adrenalectomized controls, the range was 21 to 80 minutes with an average of 41 minutes. If four controls in which the pressure remained elevated more than 45 minutes were excluded, the average would be reduced to 32 minutes. The type of anesthetic used—barbiturate or urethane—did not seem to affect the results materially.

It must be recognized that the return of blood pressure to prestimulation level does not signify exhaustion of vasoconstrictor effects. A marked drop in pressure was observed in several cases on cessation of stimulation

which had been carried on as long as 20 minutes after the return of the blood pressure to prestimulation level. Observations on the denervated n.m. were of little value since contracture occurred in most cases.



a b

Fig. 1. Nictitating membrane and blood pressure responses in a healthy, adrenalectomized, cortin-treated cat (cat 4, table 1, series A) 25 days after adrenalectomy. Urethane, 1 gram per kgm., intravenously. Upper tracing, carotid blood pressure; lower tracing, denervated n.m.—irregularities due to eye movements.

(a) *R.Spl.*, peripheral cut end of right splanchnic nerve stimulated in abdomen.

(b) *R.Sci.*, central cut end of right sciatic nerve stimulated.

2. *Examination of Cats in Adrenal Insufficiency.* (a) *Effect of brief stimulation of sympathetic nerves.* (i) *Before the development of vascular collapse.* The n.m. response to stimulation of a splanchnic nerve in seven animals examined in either a mild or moderately severe state of adrenal

insufficiency was diminished. The response was reduced absolutely as to magnitude and also relatively with respect to the epinephrine responses. The rise in blood pressure (local sympathin effect), on the other hand, was not significantly less than that found in healthy adrenalectomized, cortin-treated controls. It averaged 41 mm. Hg (table 2, cats 15 to 21). The diminution of the n.m. response in these animals was not directly correlated to the severity of insufficiency nor to the degree of pressor effect obtained

TABLE 2

Cats in adrenal insufficiency: The effect of epinephrine injections and of sympathin on the contraction of the denervated nictitating membrane

CAT NUMBER	DAYS OFF CORTIN	SIGNS OF ADRENAL INSUFFICIENCY AT TIME EXPERIMENT BEGUN				INITIAL B.P.	DEGREE OF ADR. INSUFF.	RESPONSE OF N.M. TO: EPINEPHRINE (1.0γ)			RESPONSE OF N.M. TO SYMPATHIN AS PER CENT OF AVERAGE OF RESPONSE TO EPINEPHRINE
		Strength	Weight loss	Rectal temperature	Serum K			Before	Sympathin R. splan.*	After	
			per cent	*°C.*	*mgm. per cent*	*mm. Hg*		*mm.*	*mm.*	*mm.*	
15	10	Not weak	5	36	21.5	170	Mild	65	2	72	
16	9	Weak	6	33	31	130	Mild	23§	0		
17	9	Weak	14	31.5		116	Moderate	52	19	62	33
18	10	Weak	14	33.8	26	112	Moderate	21	14	20	68
19	9	Prostrate‡	12	31.8	27	100	Moderate	26	10	22	42
20	10	Very weak	10	34		80	Moderate	35	6	10	27
21†	10	Weak	13	34.8	28	80	Moderate	16	6	10	45
22	9	Weak	3.3	34.2	29.5	80	Severe	25	0		
23	12	Weak	15	34.4	27	65	Severe	7§	0		
Average											43

Note: Blank, not done; 0, no response.

* Right splanchnic nerve stimulation for 30 seconds. Blood pressure rise resulting in cats 17–21 (moderate adrenal insufficiency) averaged 42 mm. Hg; in cats 22 and 23 (severe), 0 and 20 mm. Hg respectively.

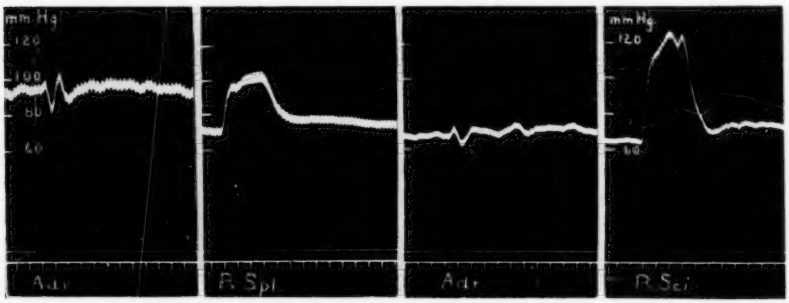
† See figure 2.

‡ Probably hypoglycemic.

§ To 0.1γ.

by splanchnic stimulation. Five of the cats (nos. 17 to 21) which showed moderately severe adrenal insufficiency are of particular interest. In all these the injection of epinephrine was repeated after the period of splanchnic stimulation in order to control the possibility that the response of the n.m. might deteriorate. In the animals with a blood pressure of 100 mm. Hg or lower (nos. 19, 20 and 21), deterioration in the response of the n.m. to epinephrine occurred. In such animals the average of the contractions

produced by the first and second epinephrine injections gives a more accurate standard with which to compare the n.m. response occurring after splanchnic nerve stimulation. This is expressed as a per cent of the average of the epinephrine responses in the last column of table 2. The average of the assays of sympathin on these animals showed that the sympathin reaching the n.m. was sufficient to cause but 43 per cent of the response shown by the cortin-treated controls (series A, table 1). The pressor response elicited by splanchnic stimulation in these animals averaged a rise of 42 mm. Hg compared to an average of 44 mm. Hg found



a b c d

Fig. 2. Nictitating membrane and blood pressure responses in an adrenalectomized cat (cat 21, table 2) in moderately severe adrenal insufficiency 10 days after cessation of cortin injections. Urethane, 0.53 gram per kgm., intravenously. Upper tracing, denervated nictitating membrane (magnification 15); lower tracing, carotid blood pressure.

(a) *Adr.*, epinephrine, 1.0 γ injected intravenously.

(b) *R.Spl.*, peripheral cut end of right splanchnic nerve stimulated in abdomen 21 minutes after epinephrine injected.

(c) *Adr.*, epinephrine, 1.0 γ, injected intravenously 21 minutes after right splanchnic stimulation.

(d) *R.Sci.*, right sciatic nerve (central) cut end stimulated 10 minutes after last injection of epinephrine.

in the cortin-treated controls (series A, table 1). Figure 2, a, b and c, illustrates these points; in figure 2d the response following sciatic nerve stimulation is shown.

(ii) *After the development of severe vascular collapse.* No n.m. response was observed following stimulation of a splanchnic nerve in cats 22 and 23 (table 2). No pressor response to splanchnic stimulation occurred in one, and a rise of but 20 mm. Hg in the other. The blood pressure was then below 30 mm. Hg. A detailed description of the course of the experiment is necessary to understand these results.

Epinephrine was injected shortly after the beginning of the experiment

when the blood pressure was still at the initial level recorded in table 2. This produced a contraction of the n.m. in both cats and also appeared to precipitate a fall in blood pressure since, during the contraction of the n.m., the heart slowed abruptly, the pulse pressure decreased and the blood pressure fell. In cat 22 this fall was from 80 to 26 mm. Hg, and in cat 23 from 65 to 28 mm. Hg. Splanchnic stimulation was done as quickly as possible—within a few minutes of the epinephrine injection—yet no n.m. effect was obtained in either cat; no pressor response in cat 22 and only a 20 mm. rise of Hg in cat 23. No n.m. or blood pressure response occurred on stimulation of a sciatic nerve in either animal.

(b) *Effect of prolonged stimulation of sympathetic nerve.* Prolonged stimulation of the right splanchnic nerve was undertaken in four experi-

TABLE 3

Effect of prolonged splanchnic nerve stimulation on the nictitating membrane and blood pressure in adrenal insufficient cats

CAT NUMBER*	DURATION OF ELEVATION OF B.P. ABOVE PRESTIMULATION LEVEL	NOTE ON B.P. CHANGE	DURATION OF ELEVATION OF N.M. ABOVE PRESTIMULATION LEVEL	NOTE ON N.M. CHANGE
	minutes	*mm. Hg*	*minutes*	
16	15	66–120†	16	Rose after 2½ minutes stimulation. Maximum rise 38 mm.
18	17	61–106†	11	Rose after 1 minute stimulation. Maximum rise 25 mm.
21	15+	67–102‡	9	Rose after 1 minute stimulation. Maximum rise 10 mm.
23	5½	38–64†	0	

* See table 2.

† Fell gradually.

‡ Had fallen to 80 mm. Hg when stimulation terminated at 15 minutes.

ments, in cats 16, 18, 21 and 23 (table 3). The results detailed in this table show that, even in moderately severe states of the vascular failure of adrenal insufficiency, splanchnic stimulation produced sustained effects, as judged by the n.m. and blood pressure responses. Depletion of sympathin at adrenergic nerve endings in the splanchnic region cannot be said to be very pronounced. The n.m. did not show the contracture phenomenon as in the acutely adrenalectomized controls.

Discussion. A normal liberation of sympathin appears to occur in cortin-treated cats for at least 41 days after adrenalectomy. In this series of experiments, brief stimulation of the splanchnic nerve caused comparable effects on the denervated n.m. and on the blood pressure in the cortin-treated group and in the group of acutely adrenalectomized animals used as controls. Prolonged stimulation of the splanchnic nerve caused a

pressor response of slightly shorter duration in the cortin-treated group than in the controls. This cannot, however, be held as evidence for impaired vasoconstriction in response to splanchnic stimulation. Variations in the force of cardiac contraction, in cardiac output or in tone of vessels in regions other than the splanchnic might account for the earlier return of blood pressure to prestimulation level in cortin-treated animals. The n.m. could not be used as an index of sympathin liberation, as contracture occurred. These results confirm the conclusions of Hoskins and Wheelon (1914), Gley and Quinquaud (1918) and others that the excitability of sympathetic vasoconstrictor nerves is not dependent on the presence of the adrenal medulla.

The possible relationship that adrenal cortical extract may have in the formation of a transmitter of sympathetic nerve activity has been discussed at length by Secker (1938). In view of our finding that splanchnic nerve stimulation produces a normal pressor effect in salt-maintained dogs (Cleghorn, Armstrong and Austen, 1938), it seems unlikely that it can have any profound influence on such a function.

In the study of animals in mild adrenal insufficiency (cats 15 and 16, table 2), the sympathin released into the blood stream, as signified by the n.m., was negligible. It is difficult to explain this satisfactorily since, in the cats in moderate insufficiency, contraction of the denervated n.m. was observed on splanchnic stimulation. However, the amount of sympathin liberated in these experiments (cats 17 to 21) appeared to be much less than that shown by the controls, as judged by the effect on the n.m. Since the pressor responses obtained in these animals were normal, compared to the cortin-treated controls, it seems that the amount of sympathin released and effective locally was not diminished. Two separate bits of evidence indicate that the amount of sympathin released after splanchnic stimulation in these cats was not diminished: first, the standard 1γ dose of epinephrine also gave a contraction of the n.m. which was consistently smaller than in the controls; second, we have found, in unreported experiments, that 1γ of epinephrine injected over a 30 second period gives a n.m. contraction of only about half that elicited by the same amount injected over a 10 second period. It is obvious that the concentration reaching the n.m. is important. We believe that the diminished n.m. response in these animals may be explained by the diminished circulation rate characteristic of this stage. This would result in greater dilution of the sympathomimetic substance and also result in a longer period available for the destruction of the liberated sympathin or the injected epinephrine (Bain, Gaunt and Suffolk, 1935; Gaddum, 1938). The advent of this stage is not associated with any change in blood chemistry but with a fall in blood pressure below a level of about 115 mm. Hg. Confirmation of the hypothesis that diminished circulation rate leads to the reduced n.m.

responses is contained in figure 2. Progressive impairment of the circulation rate was observed during the course of the experiment. The n.m. response to the second dose of epinephrine was smaller and showed a latent period following the injection, twice as long in figure 2c as in figure 2a.

Probably the portal circulation is slowed even more seriously than the systemic in such animals. As emphasized by Armstrong et al. (1939), the gastrointestinal viscera show intense congestion in adrenal insufficiency. This fact makes it seem likely that the time taken for sympathin released in the splanchnic region to reach the n.m. must be considerably extended and the opportunity for dilution increased. The amount of sympathin released under these circumstances, therefore, may not be reduced so much as would appear from our findings.

Cats in the stage of severel adrenal insufficiency, when the blood pressure has fallen to shock levels, show little or no pressor response to splanchnic nerve stimulation. This phenomenon has been discussed by Armstrong et al., 1939. They considered failure of ganglionic function as a cause and dismissed it. Measurement of intestinal volume has shown that vasoconstriction does occur on stimulation of a splanchnic nerve in severe adrenal insufficiency despite the absence of a rise in blood pressure (Cleghorn, Fowler and Wenzel, 1939). The blood pressure fails to rise, therefore, either because the heart cannot raise the pressure against the increased peripheral resistance or because of the flaccid state of the peripheral vascular system in regions other than that supplied by the stimulated splanchnic nerve. We believe that the former is probably the more important factor because there is evidence of cardiac malfunction in both cats and dogs dying of adrenal insufficiency, even when the animal is undisturbed. In cats, a terminal decrease in heart rate has been observed (Cleghorn, 1938). This coincides with the terminal fall in pressure. In dogs, cardiac irregularities and bradycardia are frequent late characteristics (Hall and Cleghorn, 1938).

SUMMARY

In healthy, adrenalectomized cats, maintained by cortin for 21 to 41 days, the n.m. and blood pressure responses to brief excitation of sympathetic nerves were found to be as great as in controls acutely adrenalectomized. Cats deprived of cortin for 9 or 10 days and showing signs of mild or moderately severe adrenal insufficiency showed an impaired n.m. response to brief splanchnic nerve stimulation but a normal pressor response. The standard dose of epinephrine (1γ) produced a smaller n.m. contraction in animals in moderate insufficiency than in controls. No n.m. contraction and little pressor response to splanchnic stimulation occurred when the blood pressure had fallen to shock levels. It is concluded that exhaustion of sympathin, at least in the splanchnic region, is not the cause of the vascular failure in adrenal insufficiency.

Acknowledgments. We would like to express our appreciation of the continued interest and criticism of Prof. Duncan Graham. To Prof. W. B. Cannon one of us (R. A. C.) is especially indebted for guidance in the use of the nictitating membrane. Valuable technical assistance in these experiments has been rendered by W. Cowan and R. Wilson.

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THE EFFECT OF RATTLESNAKE VENOM (CROTALIN) ON THE PLASMA HISTAMINE OF THE RABBIT

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The similarities of anaphylactic shock and histamine shock and the release of histamine during anaphylactic shock convinced some investigators that this liberation of histamine was fundamental and directly responsible for the most dramatic of the responses which follow the injection of the shock dose of antigen into the sensitized animal. The similarities of anaphylactic shock and histamine shock are especially well marked with certain species, the dog, guinea pig and rabbit. With the first two species anaphylactic shock is accompanied by a very significant increase in the histamine concentration of the blood. But, unexpectedly, the rabbit responds differently, for these animals in anaphylactic shock show no increase, but rather a decrease in blood histamine (Rose and Weil, 1939; Dragstedt et al., 1940). This decrease in histamine of the whole blood may be attributed to the characteristic leucopenia of anaphylaxis, for as Code (1937) has shown, the blood histamine resides for the most part in the leucocytes. Now the rabbit contains relatively large amounts of histamine also in its plasma. Rose and Weil report a decrease in 2 experiments and a small increase in 2 experiments in the plasma histamine of the rabbit in anaphylactic shock.

Histamine studies have also been made on animals shocked with snake venoms, including rattlesnake venom. It has been shown that the addition of rattlesnake venom to the fluid perfusing isolated guinea pig lungs leads to a release of histamine from these organs (Feldberg and Kellaway, 1937) and intravenous injection of venom into the dog results in an increase in blood histamine (Dragstedt and Mead, 1938), though in smaller amounts than in comparable conditions of hypotension in anaphylactic shock. It therefore appeared to us to be of interest to determine what the effect of rattlesnake venom (Crotalin) would be on the plasma histamine of the rabbit; whether there is an increase as in certain species, or a decrease as in acute anaphylactic shock of the rabbit.

METHODS. The rabbits were fasted for 24 hours. Blood samples (8–9 cc.) were taken by cardiac puncture. A small drop of heparin solution was placed in the syringe to prevent coagulation. The blood was im-

mediately centrifuged and 3 cc. of plasma pipetted into 7.5 cc. 10 per cent CCl_3COOH. After standing for about an hour the precipitate was removed by filtration and well washed. It was found, when the mixture was left in the refrigerator for a day or more and then filtered, that there was a considerable loss of histamine. The histamine was assayed by the method of Code (1938).

Dried rattlesnake venom (Crotalus horridus)[1] was dissolved in a 50 per cent aqueous solution of glycerol, such that 1 cc. contained 3 mgm. of the dried venom. The injections of $\frac{1}{3}$ cc. of this solution were made slowly ($\frac{3}{4}$ to 1 min.) into the ear vein of the rabbits. The results are tabulated.

TABLE 1

RABBIT NO.	WEIGHT	INJECTION	HISTAMINE		OBJECTIVE SIGNS
			Before	After	
	kgm.		γ/cc.	γ/cc.	
1	2.9	First	0.33	0.18 (12 min.)	–
2	2.85	First	0.77	0.29 (14 min.)	++
3	2.65	First	0.15	0.0 (21 min.)	
4	2.3	First	1.05	0.30 (5 min.)	++
	3.0	Second, 2 months later	0.41	0.07 (14 min.)	++
		Third, 1 day later	0.25	0.12 (17 min.)	++
5	2.5	First	0.23	0.15 (20 min.)	+
		Second, 2 days later	0.22	0.20 (25 min.)	++
6	2.0	First	0.50	0.40 (9 min.) 0.30 (60 min.)	+
		Second, 2 months later	0.58	0.16 (18 min.)	–

All our estimations have been made on plasma. It will be seen that in all experiments there is a definite decrease in the amount of histamine present in the plasma a short time after the first intravenous injection of the venom. A second injection of venom, some time later, into one rabbit produced no change in the plasma histamine. Two other rabbits receiving subsequent injections showed the same substantial decrease in the concentration of histamine as after the first injection. In most of the experiments the animals voided urine and feces, sometimes with diarrhea, a few minutes after the injection of venom; one animal, however, became incoördinate in its movements for a few minutes. Apart from this one exception the animals showed only mild signs of distress. An attempt

[1] We are indebted to our colleague, Dr. J. Markowitz, for the venom.

was made to make a rough evaluation of these symptoms, as will be seen from the table. The plasma of the blood samples taken subsequent to the injection of venom was frequently slightly hemolysed and cloudy in appearance.

SUMMARY

The intravenous injection of rattlesnake venom into rabbits produces a decrease in the concentration of histamine in the plasma of these animals. It is apparent that the rabbit possesses a mechanism for the relatively speedy removal of histamine from the blood, quite apart from leucopenia. We suggest that in Crotalin shock, and probably anaphylactic shock and related phenomena, histamine may be released as in animals of other species, but because of the efficiency of this removal mechanism (in the blood or other tissues) of the rabbit, even the normally circulating histamine largely disappears.

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THE CIRCULATION IN REST AND WORK ON MOUNT EVANS (4,300 M.)

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In a previous paper Asmussen and Chiodi (1940) demonstrated anew that the cardiac output of man in rest and during work is increased when the O_2-content of the inspired air is lowered, and evidence was brought forward to show that it was not the oxygen-lack *per se*, but the lowered arterial O_2-tension, which through the chemoreceptors of the carotid glomus stimulated the circulatory center. Emphasis was put on the possibility that the response of the body to *prolonged* (i.e., lasting more than a few hours) O_2-deficiency might differ from that described, and the works of Grollman (1930) and Christensen and Forbes (1937) were mentioned as examples of how prolonged exposure to low barometric pressure causes changes in the circulation rate, which cannot be explained by the stimulating effect of the low arterial O_2-tension alone. These authors found that although individual differences were apparent in their subjects, the general feature was an increased cardiac output in the first few days of an exposure to low O_2-tension, followed later by a decrease, which brought back the cardiac output practically to sea-level values.

Grollman correlated this return with the concomitant increase in O_2-capacity of the blood. However, an inspection of his data and curves shows that the greatest increase in O_2-capacity occurred during the first three days, as had been observed by Schneider (1921), whereas the decline of the cardiac output began after five or six days. In order to find an explanation of the different levels of the cardiac output in the first days and in the later stages of a sojourn at high altitude, we have studied both the percentage and total quantity of hemoglobin in the blood, the blood volume and the composition of the alveolar air. The cardiac output was determined not only in rest but also during work of known intensity. It seemed likely that such data might add useful facts to our knowledge of circulatory regulation.

LOCALITIES AND SUBJECTS. The University of Denver has for several years had a laboratory building at the top of Mt. Evans, Colorado (4,300 m.), which we were enabled to use through the kind coöperation of Dr. J. C. Stearns, Professor of Physics at the University of Denver.

The barometric pressure there during the first two weeks of August, 1940, was about 460 mm. Outdoor temperatures ranged from a minimum of 0° in the early morning to a maximum of about 20° in the afternoon. We arrived at Mt. Evans August 3 in the late afternoon and started our experiments next morning. One of us (F. C., aged 27; weight, 86 kgm.; height, 172 cm.) came directly from Boston by train. E. A. (aged 33; weight, 68 kgm.; height, 171 cm.) had spent four days in Denver (elevation 1,700 m.) and one night in Idaho Springs (elevation 2,300 m.), and furthermore had paid a short visit to Mt. Evans on August 2. Both subjects were in good physical condition upon arrival, but experienced mountain sickness during the first 48 hours. Sleeplessness, vomiting, diarrhea, precordial pains, palpitation, etc., at night were followed by a slight headache during the day. The rest of the time both were in good health, except during the last three days when E. A. began to develop a gastro-intestinal ailment, which however did not become pronounced until after leaving the mountain.

PROCEDURES AND METHODS. The general scheme for the day was: Experiments on both subjects (fasting) from about 8 a.m. to 1 p.m. Lunch and rest to about 2:30 p.m. Analysis and other laboratory work to about 6 p.m. An outdoor walk or climb to about 7 p.m. Supper and rest to 8 p.m. Calculating, dishwashing, reading, etc., to about 10 p.m. All determinations thus were made in the fasting state.

Metabolism in rest and during work was determined from the ventilation, measured on an accurate dry gas meter, and from the composition of the expired air, drawn from a mixing chamber between the valve and the gas meter. Alveolar air samples were taken in rest by the Haldane-Priestley method, during work as samples from the expiration chamber of the valve at the end of each expiration. All samples were analyzed on the Krogh-Haldane apparatus.

Cardiac outputs were estimated from the metabolic rate and the arteriovenous O_2-difference, determined by the Grollman method. As in previous experiments (Asmussen and Chiodi, 1940), we have chosen to use only those determinations in which the average O_2-tension during the rebreathing comes within a few millimeters Hg of the previously determined alveolar O_2-tension. Pulse rates were counted on the wrist and blood pressures (taken only in rest) measured in the ordinary way on the left arm.

For the determination of oxygen capacities blood was drawn from a cubital vein, saturated with O_2 in a tonometer at room temperature and analyzed in the Van Slyke manometric apparatus. The blood volume was estimated by the CO method: A measured volume (usually 170 cc. at 760 mm. Hg, 0°, dryness) of CO was mixed with O_2 and a little N_2 in a small rubber bag and rebreathed for 20 minutes, the CO_2 being absorbed in soda-lime. A sample of blood was then drawn from the cubital vein and

analyzed for CO after Van Slyke and Neill (1924). Used in this way the CO-method gives good checks with the dye method (Evans' blue) as ordinarily used in this laboratory. Its advantage is that it is very simple and can be used every day, as the elimination of the CO in our experience seemed to be complete in 24 hours, whereas the dye persists in the plasma for 10 to 12 days. From the arterial O_2-capacity and the blood volume the total O_2-capacity could be calculated.

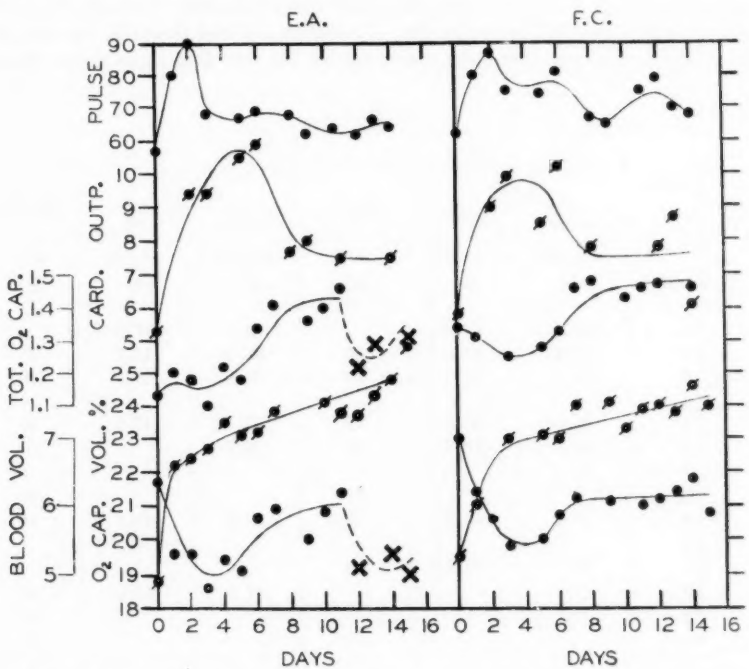

Fig. 1. Pulse rate, cardiac output (barred dots), total oxygen capacity, oxygen capacity in volumes per cent (barred dots), and blood volume for E. A. and F. C. during 15 days at 4,300 m. Crosses represent abnormal values of blood volume and total oxygen capacity (see text).

For the working experiments a simplified Krogh bicycle ergometer was used (Christensen and Forbes, 1937). Two grades of work were investigated, a lighter work (330 mkg./min.) and a heavier work (490 mkg./min.). A metronome secured a constant rhythm of 60 revolutions per minute.

Results. The results of the resting experiments on E. A. and F. C. are shown in figure 1. The dots represent the actual determinations, whereas the curves are drawn so as to smooth out the daily irregularities. In

E. A. the blood volume—and consequently the total O_2-capacity—on the 12th day for an unknown reason dropped down to values almost as low as in the first days. These values, which might have been caused by a developing ailment, as mentioned above, are shown by crosses instead of dots. We did not get any values of the cardiac output on the first day because of difficulties in finding the right acetylene-oxygen mixture. For the following days a marked initial increase in the cardiac output and a later secondary fall to values still significantly higher than the sea-level values was found in both subjects. It will be noticed that the O_2-capacity in volumes per cent increases very rapidly the two first days, later slowly

TABLE 1

DAYS AT 4300 M.	E. A.			F. C.		
	Alveolar		A.-v. O_2-difference	Alveolar		A.-v. O_2-difference
	pCO_2	pO_2		pCO_2	pO_2	
	mm. Hg	*mm.* Hg	*cc./l.*	*mm.* Hg	*mm.* Hg	*cc./l.*
(Sea-level)	42	96	47	43	101	50
1	29.1	44.3		33.1	37.9	
2	28.2	45.0	30	30.8	43.7	33
3	30.6	44.3	30	31.6	46.1	30
4						
5	29.0	50.3	27	33.4	43.9	35
6	29.8	47.3	26	29.7		29
7						
8			37			38
9	28.5	49.9	35	29.4	49.4	41
10						
11	28.1	49.8	38			
12				27.6	49.6	38
13						34
14			38			45

but steadily, whereas the *total* O_2-capacity seems to be rather stationary, or even falling a little, the first 5 or 6 days, after which it starts increasing with the above-mentioned exception for the last few days in E. A. The blood volume was markedly below sea-level values on the first day and continued to decrease for two days more. While it then began to increase it did not attain, even in F. C., who remained in good health, the sea-level value. While the pulse rate, as shown in figure 1, shows considerable changes in the first days, the blood pressure was constant and showed no significant differences from the sea-level values. Table 1 contains some additional data. It will be noticed that the alveolar pO_2 increases slightly and the alveolar pCO_2 decreases slightly during the sojourn at Mt. Evans,

indicating that the resting ventilation was increasing slightly during the process of acclimatization.

The resting metabolism was constant from day to day but, especially in E. A., somewhat increased as compared with the sea-level values. This may depend on the rather low temperatures in the morning, when the determinations were made, and therefore is more pronounced in E. A., who is rather lean, than in F. C. The possibility that the increased ultra-violet radiation, as mentioned by Lindhard (1910), might be another reason for this cannot, however, be neglected. The average values were for E. A., 283 cc./min., for F. C., 297 cc./min., compared with respectively 250 and 289 cc./min. at sea-level.

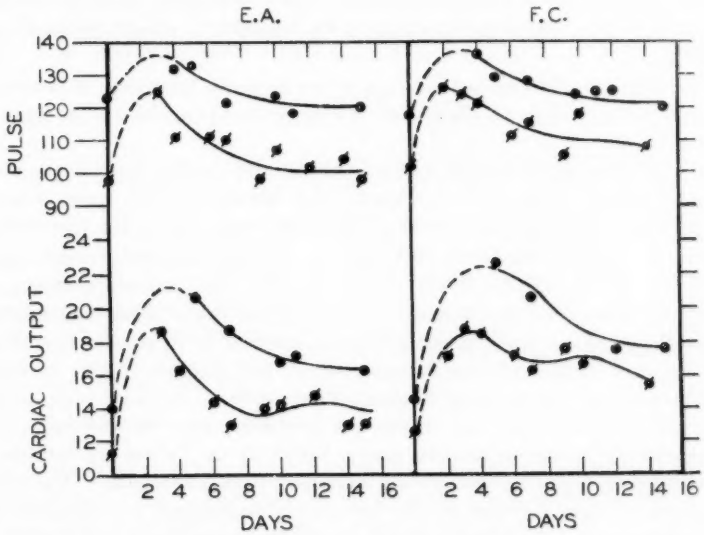

Fig. 2. Pulse rates and cardiac outputs for E. A. and F. C. in two grades of work during 15 days at 4,300 m. Dots: 490 mkg./min. Barred dots: 330 mkg./min.

The pulse rates and cardiac outputs for the two grades of work are shown in figure 2. The corresponding average metabolisms are, for E. A. 1.11 l./min. in the lighter work, 1.44 l./min. in the heavier work, for F. C. resp. 1.23 l./min. and 1.61 l./min. The efficiency of E. A. thus was higher than that of F. C. These values correspond closely to the sea-level values for both subjects. The ventilation in the steady state of work increased in both subjects during the process of acclimatization, and corresponding changes in the composition of the alveolar air were observed.

Discussion. The results of our resting experiments show qualitatively great resemblance to those of Grollman (1930). Quantitatively, however,

our results differ from Grollman's. Whereas the maximal increase in Grollman's two subjects was less than 50 per cent and the final value reached was the normal sea-level value, both of us showed an initial increase of about 100 per cent, and the final values reached were still significantly higher than our sea-level values. The only possible explanation seems to be that the discrepancies must be due to individual differences.

The decline in cardiac output after a certain time spent in high altitude has been correlated by Grollman with the well-known increase in O_2-capacity of the blood which occurs during a sojourn in high altitude. Grollman's figure 1 and our figure 1, however, show that although the greatest increase in O_2-capacity takes place in the first 1 to 2 days, the cardiac output nevertheless continues to rise thereafter, and the decline starts at a time when the increase in O_2-capacity is relatively slow and uniform.

The variations in the cardiac output correspond, however, in both our subjects rather closely to the observed changes in blood volume, as figure 1 shows.

An interdependence between blood volume and circulation rate has been asserted before. Thus Scott, Bazett and Mackie (1940) have demonstrated that during the first few days of a sojourn in a hot room the cardiac output is slightly increased, but that it assumed normal values again when the blood volume later was increased. The interdependence of the blood volume and the cardiac output is apparently a rather slow adjustment: When blood pools in the lower extremities, as in passive standing, an increased cardiac output is not evoked, but frequently the opposite (Asmussen, Christensen and Nielsen, 1939, 1940). However, for longer exposures—several hours or days—the problem must be different: the compensatory vasoconstrictions must build up a "want for oxygen," which no doubt will be met by an increased circulation rate. The very high cardiac output found in the first week thus might be explained as partly due to the low blood volume during the same time, and partly to the low O_2-tension. When the blood volume and the total Hb of the blood again increase, a decrease of the cardiac output can be tolerated and a new level is obtained.

The question now is whether the decline in blood volume (plasma volume) should be looked upon as a failure of the organism to maintain a status quo, or whether it rather should be looked upon as a regulatory device. For the former point of view speaks the fact that both of us were mountain-sick the first night and day and probably lost some water through vomiting and diarrhea. But then again it should have been easy to regain this loss of water during the following days, when no ailment was present. As this did not happen we are inclined to believe that the second suggestion is the more likely and to assume that the decline in plasma volume is a

regulatory mechanism by which the O_2-capacity of the blood is increased. This interpretation of the event has been given first by Abderhalden (1896), later by Douglas, Haldane, Henderson and Schneider (1913). The subsequent increase in blood volume is, as a simple calculation will show, for a great part, due to the increased number of erythrocytes. The significance of a high O_2-capacity of the blood for maintenance of a normal capillary O_2-tension has been discussed by Barcroft (1925) and Haldane and Priestley (1935) and needs no further explanation.

The level reached after these initial stages is in our case higher than the sea-level values. It appeared to us to be significant to determine whether in this state the usual controller of the cardiac output in low O_2—i.e., the arterial O_2-tension—was still active. We therefore in the last two days at Mt. Evans, in a few experiments on both subjects (1 during light work and 2 in rest), added O_2 to the inspired air to such a degree

TABLE 2

STATE OF SUBJECT	E. A.				F. C.			
	A.-v. O_2-difference		Cardiac output		A.-v. O_2-difference		Cardiac output	
	Rest	Work	Rest	Work	Rest	Work	Rest	Work
Acclimatized at 4300 m. (alveolar pO_2 = 50 mm. Hg)...	38	88	7.4	12.9	39	80	7.6	15.5
Acclimatized at 4300 m. (alveolar pO_2 = 150–200 mm. Hg).....	60	116	4.7	9.8	64	127	4.6	9.8
Sea-level (alveolar pO_2 = 100 mm. Hg).....	47	99	5.3	11.5	50	97	5.9	12.8

that the alveolar pO_2 increased from the normal, about 50 mm. Hg, to 150–200 mm. Hg. After 20 to 30 minutes' O_2-breathing, the cardiac output was determined. Table 2 shows the results.

The decrease in the cardiac output when the alveolar pO_2 was increased shows that the chemoreceptors must have been stimulated by the prevailing low O_2-tension also in the acclimatized subjects.

Still another observation can be made from the data of table 2: The cardiac output during the O_2-breathing was lower than in the same subjects at sea-level (compare Grollman (1930) after descent to Manitou). A supernormal alveolar O_2-tension in subjects at sea-level has no effect on the circulation rate (Grollman, 1932, confirmed by experiments in this laboratory). The difference between a subject acclimatized to high altitude and one acclimatized to sea-level, both placed at sea-level, is therefore comparable to the difference between a normal subject and a patient with anemia, both at sea-level O_2-pressure. H. E. Nielsen (1934)

has followed the recovery of a patient with pernicious anemia through two months, and found that the cardiac output gradually decreased to normal values as the O_2-capacity gradually increased. Christensen (1937), from H. E. Nielsen's results, has calculated the average venous O_2-tension, which—according to many others (e.g., Barcroft, 1925; Haldane and Priestley, 1935)—approximates the average O_2-pressure in the tissues, and points out that this is kept practically constant with Hb percentages ranging from 36 per cent to 82 per cent. Grollman (1932) likewise points out that in his experiments the venous O_2-tension in low O_2 is approximately normal, and the results of Asmussen and Chiodi (1940) confirm this. The maintenance of a constant venous O_2-tension, indicating a constant tissue O_2-tension, seems therefore to be a very important function of the circulatory adjustments.[1]

The results of our experiments in work (fig. 2) need no long discussion. The general trend seems to be the same as in rest, so that the working experiments can be said to confirm the conclusions drawn from the resting experiments.

Our results emphasize the complexity of the regulation of the cardiac output. Besides the well-known rôle of the chemoreceptors and the pressoreceptors, the capillary pO_2, the O_2-capacity, the blood volume, etc., are important. Even though their effect may be slower and therefore less striking than that of a lowered arterial pO_2 it may be as important. A more complete study of their interrelation with the circulation may give valuable information concerning the regulation of the circulation rate.

SUMMARY

Two subjects were studied in rest and work during a sojourn of 15 days at the summit of Mt. Evans, Colorado (elev. 4,300 m.). In both subjects an increase in the resting cardiac output of about 100 per cent occurred during the first 4 to 5 days, later followed by a return to values still well above sea-level values.

The blood volume decreased considerably the first 3 or 4 days, then increased to a level still below the sea-level values. The percentage O_2-capacity was increased already the first day, presumably as a result of withdrawal of fluid from the blood. Determinations of the total O_2-capacity showed that an increased formation of red cells first occurred after a latency of 4 to 5 days.

In work the cardiac output was considerably higher the first days than

[1] The experiments of Asmussen and Chiodi (1940) show that *acute* (about 1 hour) exposures to low tissue O_2-tensions, as in CO-poisoning, do not increase the cardiac output. Whether a prolonged CO-poisoning would do so still has to be proved, but seems likely considering the increased cardiac output of patients with pernicious anemia.

later on, but even then it was still higher than at sea-level. The decline in cardiac output after about a week at high altitude is presumably related to the concomitant increase in blood volume and total O_2-capacity.

The chemosensible reflexes were active during the whole sojourn at Mt. Evans. If the acclimatized subjects breathed an air mixture enriched with O_2, the cardiac output both in rest and during work fell to values lower than the corresponding sea-level values.

The findings are discussed and brought into relation with other results on the regulation of the circulation.

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THE REVERSIBILITY OF pH EFFECTS ON THE O_2 CONSUMPTION OF TISSUES[1]

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In an earlier communication (1939) we have recorded observations of the effect of changes of pH on the in-vitro O_2 consumption of certain isolated tissues. Briefly, from this work it appeared that the oxygen uptake of guinea-pig liver, kidney and testis was constant over a wide range of pH, far beyond the limits of viability of the organism as a whole. In brain the curve showed less of a plateau, and there was indeed a rather sharp maximum of oxygen consumption at about pH 9.0 to 9.5. Beyond the ranges of pH at which the oxygen uptake of the tissues was normal or above normal, the Q_{O_2} fell more or less sharply to zero, at approximately pH 4 on the acid side and between pH 10 and 11 on the alkaline side. The implications of these results, particularly in respect of the relation of aerobic oxidations to viability, made it of interest to investigate the effects of changes in pH further. The present communication deals with the reversibility of the pH effects in different tissues.

METHODS. The methods employed were similar to those of the paper referred to above. The medium was in all cases horse serum, made almost bicarbonate free by shaking with HCl at about pH 6.0 to 6.5, and adding either HCl or NaOH to bring to the desired pH. The pH of the medium was determined by means of a glass electrode before the tissues were added to the respiratory flasks, at the end of one hour of tissue respiration, and at the end of the experiment. In some experiments both the control tissues which had been respiring at physiological pH and those at test pH were transferred to other flasks containing the medium at physiological pH. In the majority of experiments a further control was carried out, namely, that at the end of an hour about one-half of the tissue at the test pH was transferred to other flasks containing medium at physiological pH, as above, the remainder continuing to respire at the test pH. These

[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

[2] We wish to record our lasting sorrow at the death of our friend and colleague, Albert Warren Stearns, Jr., during the course of this work. D. R., A. C., M. G.

manoeuvres permitted us 1, to compare the initial respiration at control (physiological) pH and at the various test pH's; 2, to determine the relative change in Q_{O_2} as a function of time at the various pH levels; 3, to determine whether a tissue which had been transferred from a medium at a test pH to one at physiological pH had a higher O_2 consumption than it had originally had at the test pH (this we have designated "absolute recovery") or whether it had a higher O_2 consumption than it would have

TABLE 1

Guinea-pig kidney

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.			
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3	(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
						per cent	*per cent*
17.5 ±0.1	16.6 ±0.1	4.3	6.2 ±0.6	5.6 ±0.3	7.3	118	130
22.3 ±0.6	20.2 ±0.1	4.5	4.7 ±0.4		4.0 ±1.0	85	
15.0 ±0.1	15.4 ±0.2	4.7	9.1 ±0.1	6.4 ±0.1	10.4 ±0.4	114	163
19.1 ±0.8	17.7 ±0.0	4.9	9.8 ±0.5		13.0 ±0.3	133	
17.3 ±1.7	16.6 ±1.0	5.2	13.7 ±1.3		15.5	113	
15.8 ±0.1	15.1 ±0.2	5.2	11.4 ±0.4	10.0 ±0.0	12.6	111	126
19.7 ±0.8	16.5 ±0.6	5.6	15.1 ±1.2		16.9 ±0.7	112	
15.4 ±0.8	14.4 ±0.2	5.8	13.0 ±0.4	11.9 ±0.4	14.4 ±0.8	111	121
20.7 ±1.7	19.5 ±0.1	6.1	16.6 ±0.8		20.2 ±0.9	122	
18.9 ±0.8	17.6 ±1.0	6.1	15.4 ±1.0		20.6 ±1.5	134	
19.4 ±1.2	18.5 ±0.4	6.8	18.8 ±1.0		19.9 ±1.2	106	
18.7 ±1.1	16.7 ±0.7	8.6	18.2 ±0.3		16.8 ±0.2	92	
15.8 ±0.1	15.1 ±0.2	8.8	15.3 ±0.3	14.3 ±0.6	13.0 ±0.1	85	91
15.4 ±0.8	14.4 ±0.2	9.3	14.9 ±0.0	13.9 ±0.3	12.1 ±0.4	81	87
18.3 ±1.0	16.4 ±0.3	9.3	12.8 ±0.5		12.6 ±0.3	98	
15.0 ±0.1	15.4 ±0.2	9.7	13.6 ±0.3	11.6 ±0.4	11.6 ±0.3	85	100
16.7 ±0.2	15.8 ±1.0	10.0	4.8 ±0.1	2.8 ±0.3	3.4 ±0.3	71	121
19.6 ±0.5	18.1 ±1.0	10.1	3.4 ±0.5		0.4 ±0.1	12	
17.5 ±0.1	16.6 ±0.1	10.2	7.5 ±0.1	2.4 ±0.7	5.0 ±0.4	67	208
16.7 ±0.2	15.8 ±1.0	10.3	3.8 ±0.5	1.7 ±0.1	2.0 ±1.0	53	117

had if it remained at the test pH (this we have designated "relative recovery").

As in the experiments previously reported a correction was made at pH's above 9.5 for the oxygen consumption of the serum medium itself, though this is not great until about pH 10.5 is reached.

RESULTS. 1. *Kidney.* The experiments on this tissue are summarized in table 1. On the acid side of neutrality, experiments were carried out over a range extending to pH 4.3. At all pH's tested below 6.8, the Q_{O_2} at the test pH was lower than the normal at physiological pH, and became

progressively less as the acidity of the medium was increased. The effect of time on this phenomenon was negligible, as the diminution of the O_2 uptake during the second hour was no greater than in the normal controls. However, that part of the tissue which was removed at the end of the first hour from the acid medium, and put into a medium at physiological pH, showed unmistakable recovery. It will be seen in table 1, in the column headed "relative recovery," that the Q_{O_2} of this transferred tissue was greater than that of the same tissue remaining at the test pH, as will be seen from the fact that the ratio (c)/(b) is always greater than 100 per cent. Not only, however, was there an arrest of the deterioration process when the tissue was transferred to the medium at physiological pH, but there was definite evidence of a reversal of effect, for as will be seen from the column headed "absolute recovery," the O_2 uptake of the transferred tissue was constantly greater than the original O_2 uptake at the test pH, as indicated by the fact that the percentile ratio (c)/(a) was consistently greater than 100. Indeed, as will be observed in the table, the respiration when the tissue was returned to the normal medium was in several cases higher than that of the same tissue that had remained in the normal medium throughout the experiment. We are therefore confronted with a phenomenon which in this case is definitely reversible.

At alkaline pH's this is not true. As we have previously shown, there is no appreciable effect, initial or otherwise, until the neighborhood of pH 9 is reached. Beyond that point, such interference with aerobic oxidation as is brought about by changes in the pH of the medium seem to be permanent, in the sense that they are not reversed when a tissue is transferred to a medium at normal pH. There is, however, some evidence of "relative" recovery, that is, of arrest of the deterioration, though this arrest was at best incomplete.

2. *Liver.* While not so striking as in the case of the kidney, there was, in the experiments in guinea pig liver, again evidence that the effect of increasing the hydrogen ion concentration of the medium on the tissue respiration is a reversible one. Only at pH 4.6—the lowest tested—was the O_2 uptake of the tissue transferred from the acid medium to the one at physiological pH less than that of the tissue at the test pH during the previous experimental period, and on the average it was eight per cent higher (table 2). The change was small, and might have been questionable had it not been reasonably consistent. The "relative" recovery over the Q_{O_2} of the tissue remaining at test pH was in all cases large and unmistakable.

At alkaline pH's the evidence indicated clearly, as in the case of kidney, that the effect of decreasing the hydrogen ion concentration (which occurred at pH's above 10) was permanent. There was no sign of "absolute" recovery, and for the most part there was not even much evidence that a

return to a normal medium had any tendency to arrest the deterioration of oxidation.

3. *Brain.* This tissue, unlike both kidney and liver, showed no indications of a reversibility of the effect on oxygen uptake of lowering the pH, as will be seen in table 3. Only an arrest of the deteriorating effect on

TABLE 2

Guinea-pig liver

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.			
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3	(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
						per cent	*per cent*
6.4 ±0.1	6.6 ±0.2	4.6	3.0 ±0.3	1.3 ±0.5	2.6 ±0.3	87	200
7.6 ±0.2	8.4 ±0.2	5.2	4.8 ±0.1	3.3 ±0.2	6.0 ±0.3	125	182
9.3 ±0.1	7.4 ±0.1	5.2	4.5 ±0.5	2.5 ±1.0	4.6 ±0.1	102	184
7.9 ±0.9	6.6 ±0.6	5.4	4.2 ±0.1		4.5 ±0.3	107	
6.8 ±0.1	8.0 ±0.6	5.6	4.7	3.3	4.7 ±0.2	100	143
8.7 ±0.3		5.6	5.6 ±0.2	4.3 ±0.3	6.2 ±0.4	111	144
5.7 ±0.2	6.4 ±0.1	5.9	4.7 ±0.3		5.2 ±0.1	111	
6.1 ±0.1		6.1	4.8 ±0.2		4.9	102	
8.4	8.1 ±0.5	6.1	5.5 ±0.2		6.3 ±0.2	115	
8.5 ±0.1	10.2 ±0.1	6.3	5.9 ±0.1	4.8 ±0.3	6.1 ±0.5	103	127
9.0 ±0.1	8.2 ±0.3	6.4	6.5 ±0.4	4.4 ±0.1	7.4 ±0.2	114	168
5.7 ±0.2	6.4 ±0.1	7.9	6.5 ±0.2	6.1 ±0.4	6.1 ±0.5	94	100
8.7 ±0.3		8.5	9.4 ±0.4	9.4 ±0.4	8.8 ±0.3	94	94
9.3 ±0.4	9.1 ±0.8	9.3	10.2 ±0.7	9.3 ±0.8	8.1 ±0.4	79	87
6.1 ±0.1		9.4	6.4 ±0.2		4.4 ±0.0	69	
7.6 ±0.2	8.4 ±0.2	9.7	8.2 ±0.2	7.2 ±0.5	6.7 ±0.0	82	93
8.4 ±0.1	8.9 ±0.2	10.0	8.6 ±0.3	6.9 ±0.1	6.9 ±0.1	80	100
8.4 ±0.1	8.9 ±0.2	10.2	6.7 ±0.1	4.4 ±0.3	4.9 ±0.5	73	111
7.6 ±0.2	8.1 ±0.4	10.3	8.7 ±0.3	5.2 ±0.7	5.7 ±1.0	65	110
8.5 ±0.1	10.2 ±0.1	10.5	8.6 ±0.3	3.2 ±0.2	4.0 ±0.1	47	125
8.7 ±0.4	8.8 ±0.5	10.5	5.5 ±0.1	3.1 ±0.8	3.4 ±0.3	56	110
6.8 ±0.1	8.0 ±0.6	10.6	4.1 ±0.2	0.8 ±0.4	2.7 ±0.2	66	+
7.6 ±0.2	8.1 ±0.4	10.6	6.6 ±0.3	3.4 ±0.7	4.9 ±0.3	74	144
6.4 ±0.1	6.6 ±0.2	10.9	1.7 ±0.6	0.0	1.3 ±0.4	76	+
8.7	8.8	10.9	1.9 ±0.7	0.0	1.1 ±0.2		

returning the tissue to a normal medium, as shown in the last column of this table, is to be observed.

On the alkaline side, our previously reported experiments had shown a precipitous fall in the O_2 consumption from a level above normal to zero at about pH 10.5. In those experiments we were fortunate in being able

to record several intermediate points in this fall. In the present research this proved more difficult, but we can report two experiments in which there was no "absolute" recovery from about a 50 to 60 per cent depression of metabolism, as well as four others in which a fall to even lower levels proved to be irreversible. At the highest pH levels there was some slight evidence of arrest of the deterioration when the tissue was returned to a medium at normal pH.

TABLE 3

Guinea-pig brain

CONTROL Q_{O_2} AT CA pH 7.3		TEST pH	TEST Q_{O_2}			REVERSIBILITY	
			1st hr.	2nd hr.			
1st hr.	2nd hr.		(a) At test pH	(b) Remaining in medium at test pH	(c) Transferred to medium at ca pH 7.3	(c)/(a) "Absolute" recovery	(c)/(b) "Relative" recovery
						per cent	*per cent*
8.4 ±0.4	6.8 ±0.1	4.4	1.8 ±0		1.0 ±0	56	
9.1 ±0.1	7.5 ±0.3	5.0	2.7 ±0.1	1.0 ±0.1	1.5 ±0.1	56	150
8.0 ±0.3	6.1 ±0.1	5.6	7.1 ±0.1	3.8 ±0.2	4.6 ±0.2	65	121
6.5 ±0.3	5.6 ±0.1	5.9	5.9 ±0.5	4.0 ±0.5	5.5 ±0.2	93	138
7.2 ±0.0	5.5 ±0.1	6.3	6.2 ±0.0	5.0 ±0.1	6.2 ±0.1	100	124
8.6 ±0.0	7.0 ±0.3	8.3	8.5 ±0.1	7.8 ±0.4	7.8 ±0.0	92	100
8.6 ±0.0	7.0 ±0.3	8.6	8.0 ±0.1	7.0 ±0.2	7.0 ±0.2	88	100
6.5 ±0.3	5.6 ±0.1	9.1	7.2 ±0.2	6.7 ±0.1	6.1 ±0.5	85	91
9.1 ±0.1	7.5 ±0.3	9.4	9.5 ±1.1	8.0 ±0.6	7.3 ±0.7	77	91
8.0 ±0.3	6.1 ±0.1	9.6	9.2 ±0.5	6.9 ±0.2	5.6 ±0.3	61	81
7.2 ±0.0	5.5 ±0.1	9.7	6.8 ±0.1	4.9 ±0.1	4.3 ±0.3	63	88
8.6 ±0.0	7.0 ±0.3	10.1	3.9 ±0.6	0.9 ±0.2	3.2 ±0.7	82	+
7.6 ±0.1	5.9 ±0.1	10.1	1.1 ±0	0.3	0.3		
7.6 ±0.1	5.9 ±0.1	10.2	1.0 ±0.0	0.6	0.2		
7.0 ±0.2	6.1 ±0.4	10.3	1.9 ±0.5	0.6 ±0.3	1.0 ±0.3		
8.4 ±0.4	6.8 ±0.1	10.5	3.1 ±0.2	0.4 ±0.4	1.9 ±0.2	62	+
7.0 ±0.2	6.1 ±0.4	10.5	1.0 ±0.3	0	0.9 ±0.1		
8.4 ±0.4	6.8 ±0.1	10.9	0	0.4 ±0.4	0.8 ±0.3		

As in the case of the other tissues, there was no indication that a change in pH, even if beyond the limits of viability of the organism, would have an effect on the O_2 consumption during the second hour of an experiment, if it had not already had such an effect during the first hour. This confirms observations made in our previous paper on this subject, and tends to support the hypothesis expressed in that paper that failure of the vital functions of the organism resulting from a change in pH is not caused by interference with either anaerobic or aerobic oxidations.

SUMMARY

Observations on the reversibility of previously described effects of changes in pH on the respiration of tissues in-vitro have demonstrated the following:

On the acid side of neutrality, the diminution of oxygen consumption which occurs at pH levels beyond the viability limits of the organism is reversible in the case of guinea-pig kidney and liver even when the pH is as low as 5. In the case of kidney the recovery may be complete. This reversibility is not seen in the case of the brain. Even in the latter, there is however some tendency towards arrest of the deterioration in respiration.

On the alkaline side of neutrality, the diminution of respiration as a result of changes in pH, beginning at about pH 9.5 to 10, are irreversible in all the tissues studied, though the effects may be to some extent arrested by returning the tissues to a medium at normal pH.

REFERENCE

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